DOCUMENT RESUME

ED 170 146 SE 027 612

TITLE	Nutrient Requirements of Domestic Animals, Number 10: Nutrient Requirements of Laboratory Animals. Third Revised Edition, 1978.
INSTITUTION	National Academy of Sciences - National Research Council, Washington, D.C. Board on Agricultural and Renewable Resources.
PUB DATE	78
NOTE	105p.; Not available in hard copy due to copyright restrictions ; Small print may be marginally legible
AVAILABLE FROM	Office of Publications, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D.C. 20418 (ISBN-0-309-02767-5; $6.75)
EDRS PRICE	MF01 Plus Postage. PC Not Available from EDRS.
DESCRIPTORS	*Animal Science; Biology; *Laboratory Techniques; Medical Research; *Nutrition; *Physiology; *Rats; Research; Science Education; Scientific Research; Zoology
IDENTIFIERS	Research Reports

ABSTRACT

This report deals with the nutrient requirements of seven species of animals used extensively for biomedical research in the United States. Following an introductory chapter of general information on nutrition, chapters are presented on the nutrient requirements of the laboratory rat, mouse, gerbil, guinea pig, hamster, vole, and fishes. Each chapter gives information on fat, carbohydrates, energy, proteins and amino acids, minerals, fat-soluble vitamins, and water-soluble vitamins. (BB)

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NUTRIENT REQUIREMENTS OF DOMESTIC ANIMALS

SE 027 612

NUTRIENT
REQUIREMENTS
OF
DOMESTIC
ANIMALS

NUMBER 10

Nutrient Requirements of Laboratory Animals

Third revised edition, 1978

RAT
MOUSE
GERBIL
GUINEA PIG
HAMSTER
VOLE
FISH

Subcommittee on Laboratory Animal Nutrition

Committee on Animal Nutrition

Board on Agriculture and Renewable Resources

National Research Council

NATIONAL ACADEMY OF SCIENCES
Washington, D.C. 1978

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the Committee responsible for the report were chosen for their special competences and with regard to appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

The primary support for this study was supplied by the National Institutes of Health of the U.S. Department of Health, Education, and Welfare. Additional support was obtained from the Science and Education Administration of the U.S. Department of Agriculture and the Bureau of Veterinary Medicine, Food and Drug Administration of the U.S. Department of Health, Education, and Welfare.

Library of Congress Cataloging in Publication Data

National Research Council. Subcommittee on Laboratory
Animal Nutrition.
Nutrient requirements of laboratory animals.

(Nutrient requirements of domestic animals; no. 10)
Bibliography: p.
1. Laboratory animals—Feeding and feeds. I. Title. II. Series: National Research Council. Committee on Animal Nutrition. Recommended nutrient allowances for domestic animals; no. 10.
SF95.N32 no. 10, 1978 [SF406.2] 636.08'52'08s 78-15118
ISBN 0-309-02767-5 [636'.9]

Available from:

Office of Publications
National Academy of Sciences
2101 Constitution Avenue, N.W.
Washington, D.C. 20418

Printed in the United States of America

PREFACE

This report deals with the nutrient requirements of seven species of animals used extensively for biomedical research in the United States. In addition to fish, six species of rodents—rat, mouse, gerbil, guinea pig, hamster, and vole—are discussed. Although some of these may occasionally be treated as pets, the predominant production and importation of these species are for scientific experimentation, bioassay, and related uses.

This edition of *Nutrient Requirements of Laboratory Animals* contains information on the gerbil and vole, species not discussed in the previous edition. *Nutrient Requirements of Cats*, published by the National Academy of Sciences this year, provides the reader with current data on that species. A companion volume dealing with another species of animal used in biomedical research, *Nutrient Requirements of Nonhuman Primates*, is also available from the National Academy of Sciences.

We are indebted to many persons for reading and critically evaluating one or more of the chapters. The subcommittee is also indebted to Philip Ross and Selma P. Baron of the Board on Agriculture and Renewable Resources for their assistance in the production of this report and to members of the Committee on Animal Nutrition and reviewers for the Institute of Laboratory Animal Resources for their reviews and suggestions.

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INTRODUCTION

The objective for this revision is to provide information concerning animal nutrition and nutrition practices useful to the biomedical research community. To meet this objective, a chapter on the general aspects of laboratory animal nutrition is included. Factors such as diet preparation, stability, and storage can have profound effects on animal performance.

The recommended nutrient concentrations have been reviewed and updated on the basis of the best-available information. However, with the exception of studies involving the rat, little or no controlled research has been conducted to ascertain the quantitative individual nutrient requirements for most species of laboratory animals. The nutrient requirement recommendations in this report are either based on data from the relatively small number of studies designed to determine nutrient requirements or estimated using data from studies in which dietary nutrient concentrations produced "acceptable" animal performance. They are expressed as average requirements for "normal" animals maintained in conventional environments but may not apply to animals in germfree or specific pathogen-free environments or under experimental stress. Recommended nutrient requirements for animals involved in long-term studies are not emphasized, because only limited data concerning the requirements of laboratory animals beyond their reproductive life span have been published. The report serves only as a guide to adequate nutrition, and does not describe the exact requirements of any specific animal or animal colony.

The extensive use of the rat in metabolic studies has resulted in a relatively large volume of literature describing signs of nutrient deficiency. Therefore, detailed descriptions of these signs are included only in the chapter on the rat, but relevant descriptions are included in other chapters as needed.

Throughout the years the accepted terminology for some nutrients (particularly vitamins) has changed. Inconsistencies in the nomenclature of nutrients may appear in the text of this publication because the terminology used in original references has not been changed.

Formulations for natural-ingredient and purified diets (open formula) have been included for all species. The most widely used laboratory animal diets are closed formula, i.e., the quantitative ingredient composition is privileged information of the manufacturers. The National Academy of Sciences publishes nutrient composition data for numerous feed ingredients in the *Atlas of Nutritional Data on United States and Canadian Feeds*, therefore, those data have not been included in this publication.

Data from controlled research experiments designed to establish the minimum nutrient requirements for each species of laboratory animals are needed to increase the efficiency, and in some cases the validity, of biomedical research. Knowledge regarding the minimum requirements of specific nutrients for growth, reproduction, and maintenance of laboratory animals may provide a basis for research to improve the quality of human life. Data generated by most research projects involving laboratory animals have either direct or indirect application to humans. The inadvertent use of nutritionally deficient animals or improperly formulated diets with nutrient concentrations that are too low or too high may result in erroneous conclusions. The extrapolation of such conclusions to other animals or humans could have dire results.

GENERAL ASPECTS OF NUTRITION

Adequate nutrition may be the most important environmental factor influencing the ability of laboratory animals to attain their genetic potential for growth, reproduction, longevity, and response to stimuli. Supplying adequate nutrition for the various species of laboratory animals involves the formulation of diets with the required concentrations of approximately 50 essential nutrients and the proper management of numerous factors relating to diet quality and intake. The kind of diets, the bioavailability of nutrients, diet palatability or acceptance by animals, procedures related to diet preparation or storage, and the concentration of chemical contaminants are examples of factors having profound effects on animal performance (Clarke *et al.*, 1977; Corbin, 1976; NRC, 1976a; Navia, 1977).

FACTORS AFFECTING NUTRIENT REQUIREMENTS

Genetic and environmental factors influence the dietary nutrient requirements of laboratory animals. Species differences in nutrient requirements are indicated by the differences in recommendations in subsequent chapters. Apparent differences in nutrient requirements among strains of animals have been demonstrated for mice by Lee *et al.* (1953), Fenton (1957), Fenton and Cowgill (1947), and Fenton and Marsh (1956). The nutrient requirements for most species of domestic animals change with various stages of the life cycle (NRC, 1968, 1976b). Similar changes in nutrient requirements of laboratory animal species have been observed but have not been extensively studied. Ross *et al.* (1976) indicate that restriction of caloric and protein intake below *ad libitum* amounts may prolong life. Adjustments in nutrient concentrations, the kinds of ingredients, and methods of preparation must be considered when formulating diets for laboratory animals reared in germfree or specific pathogen-free environments (Wostmann, 1975).

CLASSIFICATION OF DIETS

Diets for laboratory animals are classified according to the degree of refinement of the ingredients.

Natural-Ingredient Diets Diets formulated with appropriately processed whole grains such as wheat, corn, or oats or commodities that have been subjected to limited amounts of refinement such as fish meal, soybean meal, or wheat bran are referred to as natural-ingredient diets. These diets are economical and widely used. Disadvantages of using them for animals involved in research include: The inability to control completely the nutrient concentrations; variation in nutrient concentration among production batches; difficulty in altering composition to study a particular nutrient; and the potential for contamination with pesticide residues, heavy metals, or other agents that might alter the response to the experimental treatment.

Purified Diets Diets formulated with refined ingredients have been designated as purified diets. For example, casein is a source of protein, sugar or starch is a source of carbohydrate, vegetable oil or lard is a source of fat, and a form of cellulose is a source of crude fiber; chemically pure inorganic salts and vitamins are added. Planned nutrient concentrations in a purified diet can be readily obtained with minimal variation among production batches of diet. Advantages of using purified diets in research are the ability to reproduce nutrient concentrations or to alter them for induction of nutritional deficiencies or excesses. The potential for chemical contamination of these diets is low. Unfortunately, they are not readily consumed by all species.

Chemically Defined Diets Chemically pure compounds, such as amino acids, sugars, triglycerides, essential fatty acids, inorganic salts, and vitamins are used to prepare these diets. These are useful in studies where strict control of nutrient concentrations is essential, but have been found to be too expensive for general use. The nutrient concentrations in these diets are theoretically fixed at the time they are manufactured, but the availability of nutrients may be altered by oxidation or interactions among nutrients.

The best diet for a particular animal colony is dependent on production or experimental objectives. In all cases the diet must be palatable to ensure adequate food consumption and nutritious to supply nutrients essential for growth and reproduction and to overcome physiological stresses or disease. It should also be free of substances or microorganisms that may be toxic or cause infection. Diets used in research also must be readily reproducible to ensure the collection of comparable data, yet formulations must be flexible, so concentrations of only single nutrients can be altered.

DIET FORMULATION

Diet formulation is a process in which feed ingredients and various vitamin and mineral supplements are blended to produce a diet with nutrient concentrations that provide required quantities of essential nutrients. Nutrient concentrations in the diet must be adjusted for losses occurring between manufacture and actual consumption and for factors that affect feed consumption. Nutrient losses occur during various feed-processing procedures, such as sterilization (Williams *et al.*, 1968; Zimmerman and Wostmann, 1963) or as a result of interactions between nutrients; minerals can catalyze the destruction of certain vitamins, particularly when vitamins and minerals are added to diets in the same premix. Compensation in nutrient concentrations of diets also must be made for factors that decrease diet consumption. Animals tend to consume the quantity of feed that meets their energy requirements, therefore, adding fat to diets will decrease feed consumption and the intake of all other nutrients. Compounds that affect flavor may alter palatability of the diet.

Ingredients used in laboratory animal diets must be of known nutrient composition (NRC, 1971), be readily obtainable, and be palatable to the species involved. More than one ingredient should be used as a source of each class of nutrients when natural-ingredient diets are formulated. This will produce diets of higher quality, because it tends to minimize the variation in nutrient composition of a single ingredient and may increase palatability.

Sources of nutrients for purified diets have been described by Navia (1977). Formulation of such diets is more complex than those with natural ingredients, because nutrients required in trace concentrations must be provided. Concentrations of these nutrients are of little concern in natural-ingredient diet formulations, because relatively unrefined feed ingredients usually contain ample amounts. Errors of omission in the formulation of purified diets are critical, because each ingredient is the only source of an essential nutrient.

The availability of the different chemical forms of nutrients is a primary concern in the formulation of chemically defined diets. For example, the L isomeric forms of amino acids occur in natural food protein. However, the D isomers of several of the essential amino acids will support growth in the rat. Of these, methionine alone appears to be as well utilized in either form (Wretlind and Rose, 1950). The D isomer of tryptophan was found to be 61 percent as effective as the L isomer for growth of rats (Oesterling and Rose, 1952). Other essential amino acids that are at least partially effective for growth when fed as the D form are phenylalanine (Rose and Womack, 1946), arginine (Winitz *et al.*, 1957), and histidine (Cox and Berg, 1934; Wachter and Berg, 1960). The D isomers of leucine (Rechcigl *et al.*, 1958) and valine (Womack *et al.*, 1957) are poorly utilized for growth. The α-keto analogs of all the essential amino acids except lysine and threonine were found to be at least partially effective for growth of rats (Armstrong and Lewis, 1950; Bubl and Butts, 1949; Cahill and Rudolph, 1942; Chow and Walser, 1974; Jackson and Chandler, 1939; Meister and White, 1951; Pond *et al.*, 1964; Wood *et al.*, 1950).

DIET PREPARATION

The efficient manufacture of natural-ingredient diets requires large capital investments for equipment. Therefore, practically all of these laboratory animal diets are commercially manufactured. Purified or chemically defined diets can be efficiently prepared in laboratories or diet kitchens with a minimal amount of special apparatus. Navia (1977) presented a detailed discussion regarding the preparation of purified diets.

Facilities All diets for laboratory animals should be prepared in facilities that are used only for this purpose and under strict rules to prevent contamination or errors in the kinds and amounts of ingredients used. Laboratory animal diets should not be manufactured or stored in facilities used for farm feeds or any products containing additives, such as rodenticides, insecticides, hormones, antibiotics, or fumigants. Areas where diets are stored or processed should be kept clean and enclosed to prevent entry of domestic or wild animals, birds, or insects. Carefully monitored programs for control of these pests should be in effect.

Preparation The preparation or manufacture of diets involves a process in which ingredients are ground into fine particles, blended in the amounts specified in the formula, mixed, made into a physical form acceptable to the species involved, and packaged for protection until used.

Feed ingredients are ground to a similar particle size so they can be uniformly blended into a homogenous mixture to prevent animals from consuming only selected ingredients and to allow manufacture of the final product into various physical forms. The particle size of ground ingredients is dependent on the kind of ingredients involved and the planned physical form of the final product.

Blending of the exact amounts of ingredients specified in the diet formula may be the most critical step in diet preparation. Errors in omitting ingredients or adding incorrect amounts will be minimized by use of check sheets to verify the kinds and amounts of ingredients added to the mixture. Ingredients used in large amounts are added directly, while those used in small amounts, such as vitamins and minerals, are added via premixes. Separate vitamin and mineral premixes are used to minimize destruction of vitamins by oxidation reactions catalyzed by minerals and to ensure that the specific concentrations of these nutrients are distributed uniformly throughout the diet. Premixes should be prepared with one of the major ingredients as a carrier such that at least 1 percent of the premix is added to the diet. The length of time a particular combination of ingredients should be mixed for maximum distribution of nutrients is dependent on a number of factors, including particle size, particle density, mixer speed, and mixer size. "Overmixing" can occur because maximum distribution of particles is obtained within a certain period. Continued mixing results in particle separation, depending on factors such as particle density, physical form of ingredients, and the susceptibility of particles to static electrical charges that can develop in mixes (Pfast, 1976).

Diets for laboratory animals can be provided in different physical forms. Criteria for selecting a particular form are the acceptance by the species involved and requirements of experimental procedures.

Meal Meal is often an inefficient form for feeding laboratory animals. It will cake under certain storage conditions, and dust may be hazardous if toxic compounds have been added. Meal diets may be required if additives or test compounds are incorporated into an otherwise complete diet.

Pelleted Pelleted diets are formed by adding heat and moisture to meal and forcing it through a die. This results in a relatively dense product, which is usually the most efficient form of feed for a large number of laboratory animal species. Pelleted diets are relatively simple to handle, store, and feed, and animals waste minimal amounts. However, feed additives or test compounds cannot be added after pelleting.

Crumbled Crumbled diets are prepared by crushing pelleted or extruded diets and screening particles to those most appropriate size for a particular age or size of laboratory animal (usually fishes). Crumbled diets offer a method of presenting small particles of diet that, theoretically, contain all dietary ingredients present in pelleted diets. Crumbled diets offer the convenience, but not the inefficiencies of diets in meal form.

Extruded Extruded diets are formed by forcing wet meal through a die under high pressure and temperature, which results in expanded products. These products are highly palatable to fishes, nonhuman primates, dogs, and cats. Other laboratory animal species waste large amounts of extruded feed, and frequent feeding is required because of the low diet density. Extruded diets fed to fish have the advantage of floating on the surface of the water where observations of feeding activity can be made.

Baked Baked diets are expensive to produce, but are used to decrease microbial populations when other methods of decontamination are impractical.

Flaked Flaked diets are prepared by mixing finely ground ingredients with water until a slurry is formed. The slurry is drum dried and the dried sheets are broken into small particles for feeding. Flaked diets are fed to many species of laboratory fish. Most flaked diets float on the water surface, allowing observation of feeding activity.

Semimoist or Gel Forms These diets are produced by adding water, agar, gelatin, or other jelling agents, to meal. These diets should be used when toxic compounds are added. They are more palatable than dry rations and allow efficient measurement of food consumption. These diets are susceptible to microbial growth and must be frozen or refrigerated, and feeding must be at frequent intervals. Large quantities are bulky and may be hard to handle.

Liquid Liquid diets for laboratory animals have been developed to accommodate specific requirements, such as filter sterilization (Pleasants *et al.*, 1970).

Storage Nutrient stability of feeds generally increases as temperature and humidity decrease. The shelf life of any particular lot of feed is dependent on the environmental conditions in storage areas. Diets stored in areas of high temperature and humidity may deteriorate within several weeks compared to a potential storage time of up to a year in a freezer. Natural-ingredient diets stored in air conditioned areas should be used within 90 days of manufacture and purified diets within 40 days. Some of the most labile nutrients are vitamin C and vitamin A. Diets stored for long periods of time or under unusual environmental conditions should be assayed for these vitamins prior to use. Diets formulated without antioxidants or with large amounts of highly perishable ingredients, such as fat, may require special handling or storage procedures.

POTENTIAL CONTAMINANTS

Biologic Contaminants

A diet is a potential source of biologic agents that may be pathogenic to laboratory animals (Williams *et al.*, 1969). Clarke *et al.* (1977) described procedures for sampling and assaying feeds for various pathogenic organisms, as well as standards regarding the number and kinds of organisms acceptable in diets. Diets for gnotobiotic animal colonies must be decontaminated. Steam autoclaving is the most widely used method of decontaminating animal diets (Foster *et al.*, 1964; Williams *et al.*, 1968). A discussion regarding the problems and nutrient losses associated with autoclaving feeds has been presented in an earlier publication (NRC, 1976a). Animal diets have also been decontaminated with ionizing radiation (Ley *et al.*, 1969) and ethylene oxide fumigation (Meier and Hoag, 1966).

Chemical Contaminants

All animal diets, particularly those produced from natural ingredients, may contain or become contaminated with various man-made or naturally occurring compounds. (Fox *et al.*, 1976; Newberne, 1975; Yang *et al.*, 1976). Decontamination procedures for these factors are difficult or nonexistent. Chemical contaminant concentrations in diets are usually low, but occasionally may be high enough to produce clinical signs of toxicity in animals. However, low concentrations may affect biochemical or physiological processes in test animals and alter experimental results.

Periodic assay for the contaminants that may interfere with results of particular studies is recommended. A change in diet or diet ingredients may be in order if unacceptable concentrations are detected. Guidelines regarding maximum acceptable concentrations of chemical contaminants commonly found in animal feed have been listed in a previous publication (NRC, 1976a).

FEEDING PRACTICES AND QUALITY ASSURANCE

Most species of laboratory animals should have diet available on an *ad libitum* basis. Diet containers should be constructed to allow animals easy access to the feed while preventing urinary or fecal contamination. Nutrient concentrations should be monitored in order to ensure diet quality. Random diet samples should be collected and analyzed for at least the proximate nutrients (ether extract, crude fiber, crude protein, ash, moisture, and nitrogen-free extract). These analyses should be in accordance with Association of Official Analytical Chemists (AOAC) methods of analysis (AOAC, 1975).

WATER

Laboratory animals should be provided with a readily available supply of fresh water, even though there is little published information regarding water requirements for most species. The practice of providing succulent feedstuffs or wet mashes as the sole source of water is discouraged. Working with mice, Chew and Hinegardner (1957) showed that restriction in water intake resulted in decreased voluntary food consumption. Dalton (1963) demonstrated a relationship between diet density and environmental temperature on water requirements of mice.

Water is a potential source of pathogenic microorganisms and it must be decontaminated for use in germfree or specific pathogen-free environments (NRC, 1976a). The potential for chemical contamination is real in many water supplies, and the degree of contamination should be determined prior to use in animal facilities. Water can also contain sufficient amounts of minerals to prevent clinical signs of deficiency when animals are fed diets otherwise devoid of specific minerals.

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NUTRIENT REQUIREMENTS OF THE LABORATORY RAT

INTRODUCTION

Definition of nutrient requirements for any animal species can be made using several different criteria, and the requirement for a given nutrient may vary with the criterion used. Growth, reproduction, behavior patterns, nutrient storage, enzyme activity, and gross and histological appearance of tissues and their content of nucleic acids and protein are the major criteria used to assess nutritional adequacy of diets. Ideal performance is not known in all cases. It has been assumed in this report, and in most assessments of nutrient requirements, that maximum performance is ideal, although this is not true by every criterion. Greatest consumption of nutrients and most rapid growth often do not correlate with longest life span and freedom from disease (Berg, 1960; Ross and Bras, 1965, 1973, 1975).

In this edition, recommendations for nutrient concentration have not been increased to allow a margin of safety for variations in dietary ingredients or in rats. The data on which the requirements are based were reported from many different laboratories that operate under varying conditions of diet mixing and storage, rat strain, handling, and so forth. The recommendations may be assumed to be adequate for rats in different laboratory conditions. However, experimental procedures and environmental conditions may alter the requirements for one or more nutrients. For example, prevention of coprophagy or maintenance of rats in germfree status may increase the requirement for nutrients supplied in part by the intestinal microflora (Hotzel and Barnes, 1966; Wostmann, 1963). The requirements have been established in rats fed diets adequate in all other nutrients insofar as is known and in rats not previously depleted of the nutrient in question; exceptions will be discussed.

Review articles covering the older literature have been cited when possible, but the reader is referred also to the previous edition for review of the literature prior to that time. The recent literature has been reviewed and articles cited that either support or change the nutrient requirements given previously. On the basis of new studies and reevaluation of older studies, requirements for essential fatty acids, lysine, potassium, selenium, vitamin A, and thiamin have been changed. Requirements for chromium, fluoride, sulfur, and folic acid have been added, and the possibility that rats require tin, silicon, vanadium, and nickel has been discussed.

In most cases, a single requirement for each nutrient has been stated that is adequate for all stages of the life cycle. Exceptions have been made for amino acids, for which lower levels are given for maintenance than for growth, gestation, and lactation. If papers cited gave intakes per rat per day, the figures have been converted to dietary content by assuming a diet intake of 15 g/rat/day. Nutrient requirements are expressed per unit of diet weight, assuming a caloric density of approximately 4 kcal/g. The requirement per unit diet weight may be altered if the caloric density is significantly different from 4.

Nutritionally Adequate Diets for Rats

Many diets for rats are composed of natural ingredients and are conveniently obtained from commercial sources. While they are useful for colony maintenance, they may not be suited to research purposes, since their nutrient composition varies as a result of variability in ingredient composition and choice of ingredients from lot to lot. An example of a satisfactory natural-ingredient diet for mice is given in Chapter 4, Table 8.

For better control of specific nutrient intake and flexibility of manipulation of diet composition, purified diets should be used, and many references to the use of such diets appear in this publication. An example of such a diet is AIN-76 (Chapter 4, Table 9). It has been shown to produce as good growth and reproduction as the natural-ingredient diet. The suitability of this diet for long-term studies has not yet been thoroughly evaluated. The high sucrose content may be associated with development of caries in some strains of rats (American Institute of Nutrition, 1977).

Growth and Reproduction

It is not possible to describe a single growth pattern or reproductive performance applicable to all strains of rats. Body weights up to 10 weeks of age in Sprague-Dawley and Fischer

TABLE 1 Average Body Weights of Sprague-Dawley and Fischer Rats

		Body Weight (g) of Rats						
Strain[a]		Age in Days:	21	28	35	56	70	84
Sprague-Dawley	Male		46	75	120	236	302	365
	Female		44	64	100	185	210	230
Fischer 344	Male		—	53	80	160	213	256
	Female		—	44	65	123	145	162

[a] Weights are averages of figures given by two different suppliers of the two strains.

rats are given in Table 1. They are averages of data from two different suppliers. The range of weights for rats of the two strains in a single laboratory is given in Table 2. The average weights in that laboratory were somewhat greater than those given in Table 1. Other strains may follow growth patterns that differ somewhat from the ones given. Average daily diet intake for Sprague-Dawley rats over this period is approximately 15–20 g in males and 10–15 g in females. Pregnancy raises the intake to about 20 g and lactation to 30–35 g.

Examples of satisfactory reproductive performance can be found in many of the references cited. Figures from two studies are given in Table 3.

FAT

Fat has been considered an optional component of the diet, except as a source of essential fatty acids (EFA) and for its role in the utilization of dietary fat-soluble vitamins. *Fat deficiency does not occur* (except for the EFA deficiency) *in the sense that a specific syndrome develops.* Certain evidence, however, suggests that some fat in addition to the EFA is desirable. After a lifetime of study in the field, Deuel (1957) was convinced that fat was an obligatory part of the diet.

There is evidence that supplementary fat is desirable for growth (Deuel *et al.*, 1955b; Greenberg *et al.*, 1950, 1951; Henderson *et al.*, 1945; Lassen and Bacon, 1949; Mohrhaurer and Holman, 1967; Pearson and Panzer, 1949) and lactation (Loosli *et al.*, 1944). Female rats reach sexual maturity earlier if diets with added fat are fed (Deuel *et al.*, 1947). Excellent reproduction and lactation have been maintained for 4 (Dryden *et al.*, 1957) to 46 generations (Alfin-Slater *et al.*, 1957) in rats fed diets that contain 10 and 11 percent fat. Differences in reproduction and lactation are small when diets contain from 3 to 18 percent fat (Richardson *et al.*, 1964). Swift and Black (1949) reported greater diet acceptance by rats fed 30 percent fat than rats fed 2 percent fat.

There also are reports in which increases in the fat content did not result in appreciable improvement in growth (Aaes-Jorgensen and Dam, 1945a,b; Barki *et al.*, 1950; Hoagland *et al.*, 1952; Meng and Youmans, 1955; Thomasson, 1955). French *et al.*, (1952, 1953) and Swift (1952) reported reduced life span and reproduction in rats fed diets that contained 20 percent corn oil compared to diets with lower concentration.

When moderate amounts of fat are added to the diet, caloric consumption is more frequently increased than depressed (Barki *et al.*, 1950; Deuel *et al.*, 1947) and carcasses tend to contain more fat (Barki *et al.*, 1950; Dryden *et al.*, 1957; Lassen and Bacon, 1949; Scheer *et al.*, 1947b). Mickelsen *et al.* (1955) induced obesity in rats by feeding 64 percent fat.

The evidence is not conclusive for establishing the optimal dietary concentration of fat. Almost any value may be derived from the literature. Barki *et al.* (1950) reported no consistent trend in growth with increasing dietary fat, but the carbohydrate source was found to be important (Boutwell *et al.*, 1943; Dryden *et al.*, 1956). Peifer and Holman (1959) have suggested that the content of EFA fed may account for varying results. Many workers have reported increasing weight gain with increasing fat content (Aaes-Jorgensen *et al.*, 1957; Barnes *et al.*, 1959; Deuel *et al.*, 1947; Hoagland and Snider, 1940, 1941; Scheer *et al.*, 1947a). Scheer *et al.* (1947a) and Deuel (1955a) recommended 30 percent fat as optimal. A limited experiment by Hartsook *et al.* (1973), which used multiple regression techniques, showed that the amounts of energy supplied in the form of fat and carbohydrate affected metabolizable energy. When the diet contained approximately 25 percent protein, decreasing the calories from fat increased the metabolizable energy; the reverse was true when the diet contained approximately 50 percent protein.

In spite of the contradictory nature of published data, a dietary standard should specify fat content. Experimental

TABLE 2 Range of Body Weights of Sprague-Dawley and Fischer Rats

		Body Weight (g) of Rats					
Strain[a]		Age in Days:	21	28	56	84	168
Sprague-Dawley	Male		50–61	71–114	206–259	317–385	436–482
	Female		45–63	71–130	188–205	231–283	275–301
Fischer	Male		24–39	28–56	155–209	183–238	312–380
	Female		22–39	35–51	105–156	112–175	203–238

[a] Poiley, S. M., 1972.

TABLE 3 Reproductive Performance in Sprague-Dawley Rats

	Does		Pups	
Reference	Fertility[a] (% ± SD)	Wt. Gain During Gestation (g)	No. Per Litter (± SD)	Birth Wt. (g ± SD)
Kirksey *et al.*, 1975	—	125–155	11 ± 2	5.1 ± 0.3
Newberne *et al.*, 1973	94 ± 7	–	11 ± 1	6.1 ± 0.3

[a] Percent bred females that produced live offspring.

diets for rats have varied widely in fat content, and the range of energy concentrations used is quantitatively significant. It has frequently been implied or stated (Crampton, 1964; Goettsch, 1948; Kleiber, 1945; Mendel, 1923; Mitchell, 1955; Wretlind and Rose, 1950) that most nutrients are consumed and used as a function of energy metabolism and that their concentration in the diet is properly related to the energy concentration of the diet. The nutrient that affects most profoundly the energy concentration of the diet is fat. With existing data, it is impossible to state with certainty how much each nutrient should be changed as the caloric density (fat percent) of the diet changes. The error will probably be small, however, if a constant nutrient-to-calorie ratio is maintained.

The following observations are used in setting a minimal level of dietary fat. Swift and Black (1949) showed that the greatest improvement in energy retention occurred when fat content was increased from 2 to 5 percent; additional increments in energy retention were smaller when fat content was above 5 percent. Deuel *et al.* (1947) reported that the greatest reduction in number of days required to reach puberty occurred when the percentage of fat in the diet was increased between 0 and 5 percent. Again, changes with increasing fat above 5 percent were relatively small. Burns *et al.* (1951) demonstrated that 5 percent fat was satisfactory for absorption of carotene and vitamin A. Loosli *et al.* (1944) reported only slight improvement in weight gain of pups when lactating mothers were fed diets that contained more than 5 percent fat. Furthermore, many fats provide ample EFA when included in the diet at these concentrations. Therefore, the desirability or need for dietary fat greater than 5 percent has not been established, and the content of fat suggested to meet the needs of all physiological activities is 5 percent.

Deuel (1955a,b, 1957) summarized the extensive literature on the relative value and digestibility of different fats.

Essential Fatty Acids

The early work of Burr and Burr (1929) demonstrated the essentiality of dietary fat. Polyunsaturated fatty acids, mainly linoleic, were shown by Burr and Burr (1930) to combat the adverse effects of fat-free diets. Of the three polyunsaturated fatty acids—usually referred to as essential fatty acids—linoleic (18:2 *n*-6), linolenic (18:3 *n*-3), and arachidonic (20:4 *n*-6), arachidonic has the highest biopotency for growth (Holman, 1968). Arachidonic acid is found predominantly in animal tissues (Haines *et al.*, 1962; Hulanicka *et al.*, 1964). Linoleic acid is widely distributed in plant oils and is the most abundant dietary EFA. Arachidonic acid itself is not essential; it can be derived from linoleic (Sprecher, 1972), γ-linolenic (18:3 *n*-6), and decosapentaenoic (22:5 *n*-6) acids (Holman, 1970). The question of the essentiality of each of these polyunsaturated fatty acids is still open. γ-Linolenic acid is found in low concentration in tissues (Holman, 1970) and is considered to derive its EFA activity as a precursor of arachidonic acid. Whether or not linoleic acid itself is essential is not clear, as it is converted to arachidonic acid but yet is found in high concentrations in hepatic phospholipids (Holman, 1970). Linolenic acid is not a precursor of arachidonic acid (Sprecher, 1972) and is thought not to be essential. Tinoco *et al.* (1971) showed that rats can be raised to the third generation without signs of deficiency when fed diets free of linolenate. Crawford and Sinclair (1972) took issue with the changes reported in tissue unsaturated fatty acid levels, because whole tissue analysis may mask alteration in membrane unsaturated fatty acid levels; but this criticism does not negate the essentially normal performance of rats fed linolenate-free diets for three generations.

Odd-chain polyunsaturated fatty acids of chain lengths 17, 19, and 21 have EFA activity (Schlenk, 1972; Schlenk and Sand, 1967). They are found in low concentration in the liver and are derived from dietary sources.

There appears to be considerable competition in metabolism of unsaturated fatty acids (Holman, 1964, 1970; Schlenk, 1972; Sprecher, 1972). In EFA-deficient rats, oleate (18:1 *n*-9) is extensively converted to the 20:3 *n*-9 fatty acid and is found in high concentration in tissue lipids. Linoleic acid (18:2 *n*-6) markedly depresses synthesis of the 20:3 *n*-9 fatty acid and is itself converted into arachidonic acid (20:4 *n*-6), which is found in high concentration in tissue lipids. This relationship is the basis of the use of the triene:tetraene ratio, which has been suggested as a means of assessing EFA deficiency (Holman, 1960, 1964, 1970).

Diets that contain more than 5 percent saturated fat may require a greater concentration of linoleate for support of maximum performance (Aaes-Jorgensen *et al.*, 1955, 1956; Kaunitz *et al.*, 1960; Peifer and Holman, 1959). This is apparently not due to a direct effect of saturated fatty acids on linoleate conversion to arachidonic acid, but probably reflects the importance of linoleate in utilization of saturated fatty acids (Holman, 1968). Oleic acid (Lowry and Tinsley, 1966) and cholesterol (Holman and Peifer,

1960) also increase linoleic acid requirements. In light of the above, expression of the EFA requirement as a percentage of calories appears to be justified.

The requirement for EFA is usually expressed in terms of linoleic acid, with the greatest biological activity attributed to the *cis–cis* isomer (Privett *et al.*, 1955, 1967). From 20 to 100 mg of linoleic acid per rat per day is required in order obtain maximum growth (Holman, 1970). From his early work Holman suggested that a ratio of triene:tetraene of less than 0.4 in the fatty acids obtained from liver, erythrocytes, and heart lipids indicates that the minimum requirement for linoleic acid has been met (Holman, 1960). In general ratios of 0.4 or less are observed when the diet contains linoleic acid at 1–2 percent of the calories. It is important to note that the triene:tetraene ratio is valid only when linoleic acid is the major polyunsaturated fatty acid in the diet. Other polyunsaturated fatty acids such as linolenic acid (18:3 *n*-3) and its metabolic products depress the synthesis of the triene fatty acid (20:3 *n*-9) and may yield a low ratio even with diets deficient in EFA.

Using these criteria, Pudelkewicz *et al.* (1968) considered the linoleic acid requirement to be 1.3 percent of the calories for males and 0.5 percent of the calories for females. Assuming that the caloric density is 9 kcal/g for pure linoleic acid and the gross energy of the diet is 4 kcal/g, the linoleic requirement can be calculated to be 0.6 percent for males and 0.22 percent for females. The requirement for reproduction is met by diets adequate for growth. The requirement for lactation is in excess of 80 mg per day and would be met by consumption of 35–40 g of a diet that contained 0.22 percent linoleic acid. A level of 0.3 percent is suggested, however, because this level of intake may not always be achieved by lactating females. A summary of these requirements is presented in Table 6.

Holman (1968) extensively reviewed the EFA literature and found that a number of factors affect development of deficiency under laboratory conditions. While commercial vitamin-free casein is sufficiently free of lipids to be used in essential fatty-acid-deficient diets, soybean and cottonseed meals extracted with organic solvents may not be free of EFA and should be used with caution to induce deficiency. Similarly, commercial starches used in the diet at concentrations of 70–75 percent may provide sufficient EFA to prevent development of deficiency. For these reasons, Holman (1968) suggests that vitamin-free casein and sucrose make up the protein and carbohydrate components of EFA-deficient diets. Other factors, such as the EFA status of the dam, age of rats fed the EFA-deficient diet, restriction of water intake, and coprophagy, affect development of the deficiency.

Signs of Deficiency

The signs of EFA deficiency in the rat (Holman, 1968, 1970) are reduction in growth, which plateaus after about 12–18 weeks; scaly skin; a rough, thin hair coat; necrosis of the tail; electrocardiographic abnormalities; fatty liver; renal damage; hematuria; and death. There is an increase in the basal metabolic rate, which may increase caloric intake and water consumption. Females manifest irregular estrus, prolonged gestation, fetal resorptions, difficult and prolonged parturition, litters of low viability, and reduced lactation; spermatogenesis is impaired in the male. In the young, skin lesions develop in 5 to 12 weeks and become progressively worse. These signs become more severe if the relative humidity is 40–50 percent or lower.

Panos and Finerty (1954), Panos *et al.* (1956), and Morris *et al.* (1957) noted that the increase in basal metabolic rate occurred during the first 2 weeks, before gross lesions were seen. Increased oxidation of TCA cycle intermediates by liver homogenates, decreased mitochondrial arachidonate, and increased succinic dehydrogenase were observed also at this time (Hayashida and Portman, 1965; Klein and Johnson, 1954; Smith and DeLuca, 1963).

The deficiency syndrome is not easily produced in adult animals, and spontaneous recovery may occur. Barki *et al.* (1947) produced the syndrome by offering a fat-free diet after a period of caloric restriction.

CARBOHYDRATES

Although previously it was thought that there was no specific requirement for carbohydrate, it is now clear that glucose or gluconeogenic precursors should be included in the diet of the rat. Recent studies show that rats cannot maintain their weight, are hypoglycemic, and have abnormal glucose tolerances and high levels of plasma insulin in response to a glucose load when they are fed diets in which 90 percent of the calories are supplied as free fatty acids and 10 percent as protein. Rats were able to gain when neutral fats were substituted for the fatty acids, but remained hypoglycemic and had an abnormal insulin response to a glucose load. When glucose or glycerol was added to the diet at a concentration equal to that of glycerol in the neutral fat, weight loss was prevented but plasma glucose was abnormal (Carmel *et al.*, 1975; Konijn *et al.*, 1970). From these studies, it appeared that rats fed these diets supplemented with 8 percent glucose had normal glucose tolerance curves. More work is required to determine if other aspects of glucose metabolism and growth become normal with this level of glucose. Rats were able to grow slowly and had normal blood glucose levels when dietary protein content of free-fatty-acid-containing diets was increased from 10 percent of the calories to 18–20 percent of the calories (Akrabawi and Salhi, 1973; Goldberg, 1971). However glucose tolerance curves were still abnormal (Goldberg, 1971). Beneficial effects of increased dietary protein content observed in rats fed *ad libitum* were abolished in rats trained to consume diets within a 2-hour period (Akrabawi and Salhi, 1973).

In rats fed diets low in protein or water-soluble vitamins, insoluble carbohydrates such as starch or dextrin promoted more growth than did soluble carbohydrates such as sucrose or glucose. It is not known how addition of insoluble carbohydrates to low-protein diets results in enhanced growth, but improved utilization of dietary protein or increased numbers of intestinal microorganisms and vitamin synthesis by them have been suggested (Harper and Elvehjem, 1957).

Increased food intake by rats fed diets that contain insoluble carbohydrates also may account for some of the effect. The interaction of gastrointestinal organisms with nonruminant animals and the effect of coprophagy on nutrition have been reviewed by Hötzel and Barnes (1966).

In limited studies of the effect of dietary fat, carbohydrate, and protein concentration on the efficiency of energy utilization by rats, Hartsook *et al.* (1973) found that the heat increment, an indicator of efficiency of energy utilization, was unaffected by wide variations in the fraction of energy supplied by fat or carbohydrate and was minimal when the protein concentration was 46 percent.

A number of carbohydrates can be used by the rat. In general, if the diet is adequate in other respects, glucose, sucrose, maltose, and fructose support similar levels of performance (Day and Pigman, 1957; Hundley, 1949; Buchmann *et al.*, 1938). Diarrhea may occur if dietary sugar content is too great. Poor performance and cataract formation occur in rats fed lactose or galactose. A number of sugars apparently are not utilized by the rat: raffinose, melibiose, "mannoheptulose," L-rhamnose, and C-xylose (Day and Pigman, 1957). Xylose is toxic (Booth *et al.*, 1953; Day and Pigman, 1957); lens opacity was observed in rats fed diets that contained as little as 15 percent D-xylose, and digestive upsets similar to those seen with lactose were observed when xylose was fed at greater concentrations.

A variety of starches are well utilized by the rat, e.g., wheat, maize, rice, and cassava (Booher *et al.*, 1951). Potato starch was less available, and Jelinek *et al.* (1952) suggested that the potato starch granule is resistant to rats' digestive enzymes.

Fiber

While there appears to be no requirement for inclusion of a source of fibrous material in the diet, it has been the usual practice to include 5 percent of a nonnutritive fiber (e.g., cellulose) because that approximates the amount found in diets made from natural ingredients (Knapka *et al.*, 1974). Excessive dilution of diets with fiber can decrease nutrient and caloric intake (see below).

ENERGY

Most purified, low-fat (5–10 percent) diets contain from 4.0 to 4.5 Mcal/kg of gross energy. The digestible energy (DE) of purified diets ranges from 90 to 95 percent of the gross energy (GE) (Deb *et al.*, 1976; Hartsook *et al.*, 1973; McCraken, 1975). The metabolizable energy (ME) varies from 90 to 95 percent of the digestible energy (Deb *et al.*, 1976; Hartsook *et al.*, 1973; McCraken, 1975; Pullar and Webster, 1974). These values may be somewhat lower when diets formulated from natural ingredients are used (Peterson and Baumgardt, 1971a; Yang *et al.*, 1969). Addition of cellulose to natural diets depresses energy digestibility (Peterson and Baumgardt, 1971a; Yang *et al.*, 1969) even though a substantial fraction (15–60 percent) of the cellulose itself is digested (Conrad *et al.*, 1958; Peterson and Baumgardt, 1971b; Yang *et al.*, 1969). Some of the depression in energy digestibility is due to the low digestibility of cellulose itself, and a portion is due to increased fecal nitrogen losses (Meyer, 1956).

In general the rat will eat in relation to its energy requirement (Brody, 1945; Kleiber, 1975; Mayer *et al.*, 1954; Peterson and Baumgardt, 1971b; Sibbald *et al.* 1956, 1957; Yoshida *et al.*, 1958). This is probably most clearly demonstrated by the proportionate increase in consumption of diet in relation to dilution with inert materials. A maximum level of 40 percent dilution of the diet could be made in weanling female rats before caloric intake was reduced, whereas 50 percent dilution could be made in mature females (Peterson and Baumgardt, 1971b). The energy requirement of the lactating female is such that dilution of the diet with 10 percent of inert material results in significant depression in digestible energy intake (Peterson and Baumgardt, 1971b). Inadequate dietary protein content may depress energy intake (Menaker and Navia, 1973). A depression in food intake would be expected when the energy content of the diet is increased. Yoshida *et al.* (1958) reported that daily caloric intake remained constant when the diet contained from 0 to 30 percent fat.

Temperature, age, and activity influence the energy requirement of the rat. The critical temperature of the fasting rat is 30°C (Brody, 1945; Kleiber, 1975; Swift and Forbes, 1939). The critical temperature is that environmental temperature below which heat production must be increased in order to maintain body temperature. The basal metabolic rate of the rat can be estimated from the general formula $B = 72W_{kg}^{0.75}$, where B is the heat production in kcal per day, W is the weight in kilograms, and 72 is the average heat production per $kg^{0.75}$ of 26 groups of rats studied (Kleiber, 1975). This formula provides a reasonable estimate of the basal heat production of mature animals; that of young rats may be 1.2 to 1.4 times that of the mature rat (Figure 1).

Maintenance

The maintenance energy need is that portion of the total energy requirement that is separate from the needs for growth, reproduction, and lactation. The maintenance energy requirement is the sum of needs for basal heat production, physical activity, alterations in ambient temperature, and those needs associated with the consumption and utilization of food energy. Animals fed at the maintenance level should maintain energy equilibrium.

An estimate of the maintenance requirement for the adult rat (300 g) expressed in terms of ME can be made by increasing the figure for basal heat production ($72\ W_{kg}^{0.75}$, Kleiber *et al.*, 1956) by approximately 20 percent (Morrison, 1968) to cover the requirement for activity and by conversion of the basal heat production, which is in net energy units, to ME units by correcting for the conversion of ME to net energy, which is approximately 75 percent efficient (McCraken, 1975). The daily maintenance energy requirement in ME units for adult rats based on these assumptions is 114 kcal of $ME/W_{kg}^{0.75}$. This value is remarkably similar

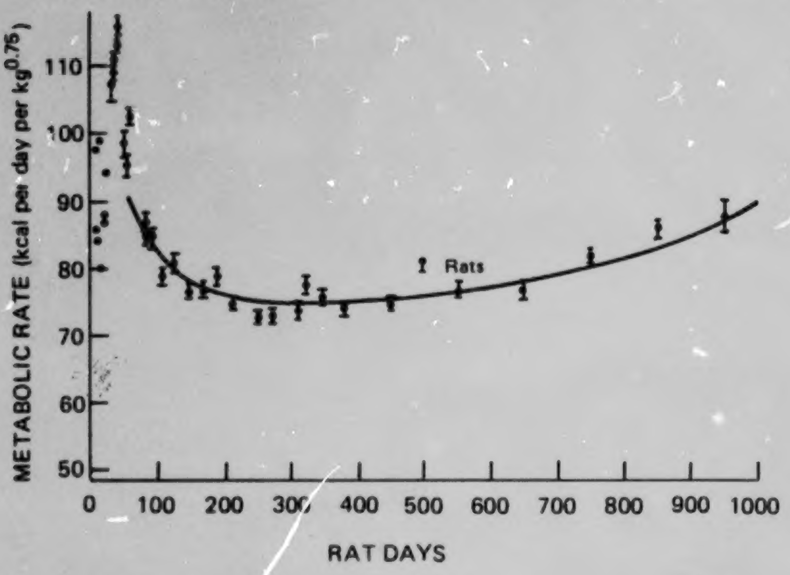

FIGURE 1 Effect of age on basal heat production of female rats in kcal/day/kg$^{0.75}$ (modified from Kleiber, 1975). The heat production of rats from 77 to 1,000 days of age can be predicted from the following equation:

$$B = 72.6W^{0.75}(1 + 0.55e^{-0.014a} + 0.008e^{0.0034a}),$$

where B is kilocalories per day, W is body weight in kilograms, a is age in days, and e is the base of the natural logarithm.

to direct estimates of the maintenance energy requirement (106 kcal/ $W_{kg}^{0.75}$) in ME units (100 kcal, Pullar and Webster, 1974; 106 kcal, McCraken, 1975; 130 kcal, Deb *et al.*, 1976; 91 kcal, Ahrens, 1967). If an average of 95 percent of the GE is recovered as DE and a similar percentage of the DE is recovered as ME, then the maintenance energy requirement in terms of GE, DE, or ME is as follows:

$$\text{GE} = 117\ \text{kcal}/\,W_{kg}^{0.75}$$
$$\text{DE} = 110\ \text{kcal}/\,W_{kg}^{0.75}$$
$$\text{ME} = 106\ \text{kcal}/\,W_{kg}^{0.75}$$

The requirement for fat rats (e.g., obese Zucker) is approximately 15 percent lower (Deb *et al.*, 1976; Pullar and Webster, 1974).

Growth

The maintenance energy requirement can be estimated with some degree of confidence; but it is much more difficult to estimate the energy requirement for growth, because energy used for growth becomes available only after the energy requirement for maintenance has been met. The problem is complicated further by variation in the composition of weight gain (Deb *et al.*, 1976; Hartsook *et al.*, 1973; McCraken, 1975; Meyer, 1958; Schemmel *et al.*, 1972) and in the energetic efficiency of net protein synthesis (43 percent) or fat synthesis (65 percent) for both lean and obese rats (Pullar and Webster, 1974).

Because the rat is able to regulate accurately its daily energy intake, its requirement for maintenance and growth can be met by diets with a wide range of energy densities (kcal/cm^3). Peterson and Baumgardt (1971b) reported that weanling and mature rats consumed 225 and 150 kcal DE/day/kg$^{0.75}$, respectively, when the energy density of the diet varied from 2.5 to 5.0 kcal DE/cm^3. When the energy density in the diet fell below 2.9 kcal DE/cm^3, the weanling rat could not meet its energy requirement, but the mature rat could meet its energy requirement until DE density fell to values below 2.5 kcal/cm^3. These levels are equivalent to dilution of the diet with 40 and 50 percent of inert material for the weanling and mature rats, respectively.

Gestation and Lactation

The energy requirement for gestation appears to be 10–30 percent greater than that of the mature female rat fed *ad libitum* (Menaker and Navia, 1973; Morrison, 1956). Food intake of rats fed diets adequate in protein increased 10–20 percent (Menaker and Navia, 1973) or 20–30 percent during the first days of gestation and up to 140 percent by the sixteenth to eighteenth day of gestation (Morrison, 1956). Total heat production in pregnant females increased approximately 10 percent above that of nonpregnant females (Brody *et al.*, 1938; Champigny, 1963; Kleiber and Cole, 1945; Morrison, 1956). Approximately one-third of the 100–200 kcal of energy stored over the gestation is recovered in the fetal tissues (Morrison, 1956). Restriction of the diet during gestation decreases the size and viability of the young and may induce resorption (Berg, 1965; Perisse and Salmon-Legagneur, 1960). Protein appears to be more critical than energy for satisfactory reproduction (Hsueh *et al.*, 1967; Menaker and Navia, 1973).

Lactating rats consume from two to four times the energy of nonlactating females (Menaker and Navia, 1973; Nelson and Evans, 1961; Peterson and Baumgardt, 1971b). Although some of the increase in measured intake may be due to consumption of diet by the litter, this is not significant until about 15 to 17 days postpartum, a time when the dam's energy requirement is decreasing because of the ability of the young to consume solid diet. Peterson and Baumgardt (1971b) reported that lactating females suckling 10 pups consumed 555 kcal DE/day/ $W^{0.75}$. This value agrees with calculations made from the data of Kennedy (1953). At this

TABLE 4 Protein Requirements of the Rat Expressed as a Ratio of Dietary Protein (N × 6.25) to Gross Kilocalories for Diets Containing Little Fibrous Material[a]

	Protein		Protein/kcal		Dietary Protein Concentration[a]	
Source	True Digestibility (%)	Biological Value (%)	Growth, Gestation, or Lactation (mg protein/kcal GE)	Maintenance (mg protein/kcal GE)	Growth, Gestation, or Lactation (%)	Maintenance (%)
Ideal protein	100	100	29	10.7	12	4.2
Casein	98[b]	90[b]	33	12.1	13.6 (15.1)[c]	4.8 (5.3)[c]

[a] See also Table 6, based on diets that contain 5 percent fat or about 4 kcal/g of diet.

[b] Figures applicable to casein properly supplemented with sulfur-containing amino acids (0.2 percent of either cystine or DL-methionine should be adequate).

[c] The amounts of casein (90 percent protein) required in diets to provide the recommended levels of net protein are 15.1 percent for growth, gestation, or lactation and 5.3 percent for maintenance, respectively (dry-weight basis).

level of intake, an energy density (kcal/cm^3) of at least 4.5 kcal DE/cm^3 must be provided for the lactating rat to meet its energy needs. Dilution of the diet by only 10 percent results in diminished DE intake. The lactating rat therefore requires a high-quality diet in unrestricted quantities.

PROTEINS AND AMINO ACIDS

Growth

In establishing the protein requirements for each of the physiological functions, three factors have been considered: (a) energy concentration of the diet, (b) amino acid composition of the protein, and (c) digestibility of the protein. Since feed intake is related to the energy content of the diet, the protein requirement is most properly expressed as a protein-to-calorie ratio (Goettsch, 1948). Ideally, the minimum net protein* requirement is expressed as the amount of protein with the proper amino acid balance used per unit of dietary net energy.

In the absence of specific data, the 1972 issue of this publication (NRC, 1972) listed a protein requirement for weanling rats that had been calculated from the literature (Barnes *et al.*, 1946; Goettsch, 1948; Hamilton, 1939; Hartsook and Mitchell, 1956; Hoagland *et al.*, 1948; Mitchell and Beadles, 1952; Rose *et al.*, 1948). A value of 29 mg of net protein per gross kilocalorie was derived, which corresponded to a low-fiber diet containing 4 kcal/g and 12 percent whole egg protein. This basic requirement is listed in Table 4 and expanded to include diets that contain properly supplemented casein. Breuer *et al.* (1963) fed a fiber-free diet that contained 5 percent fat and found that 14 percent casein (88 percent protein) supplemented with 0.18 percent DL-methionine supported gains nearly equal to those obtained with diets that contained 20 percent casein supplemented with DL-methionine. Sibbald *et al.* (1956, 1957a,b) reported that a minimum of 22 mg of apparently digested protein per kilocalorie of apparently digested energy produced the most-efficient nitrogen retention. This lower value agrees with the fact that the most-efficient storage of nitrogen occurs at a dietary content below that necessary for maximal gain (Barnes *et al.*, 1946; Bunce and King, 1969a,b; Forbes *et al.*, 1955). The data in Table 4 may be expressed in terms of metabolizable calories, assuming the diets contain 90 percent ME (Metta and Mitchell, 1954; Swift and Black, 1949).

Computation of the percentage of dietary protein required for optimal growth when the diet contains a mixture of proteins from different sources requires that consideration be given to both content and availability of amino acids in the different proteins. The proper amount of essential amino acids, the proper amount of dispensable amino acids or other nonspecific nitrogen sources, and excesses as well as deficiencies of amino acids are factors that may influence results. Methods for determining protein quality, such as amino acid index (Oser, 1959), are useful but do not account for variations in absorbability or in biological value with increasing levels of protein intake (Barnes *et al.*, 1946; Forbes *et al.*, 1958). If ingredients are selected to provide adequate amounts of essential amino acids, 15–20 percent total protein should be sufficient. In practice, natural-ingredient diets that contain 18–25 percent protein have been successful.

There is no doubt that protein requirement declines with age after weaning, but the problem has not been studied extensively (Forbes and Rao, 1959; Hartsook and Mitchell, 1956). Hartsook and Mitchell (1956) estimated by carcass analyses that the requirement declined from about 28 percent of the diet (57 mg net protein per gross kilocalorie) at 30 days of age to 10 percent (20 mg net protein per gross kilocalorie) at 50 days of age. The higher value agrees with that calculated from an analysis of rat milk (Luckey *et al.*, 1954).

In the estimation of the amino acid requirements, it is necessary to give consideration to the energy concentration of the diets used (Rosenberg and Culik, 1955; Wretlind and

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*Net protein (nitrogen) is the amount that is used for maintenance and production. The net protein (nitrogen) requirement for maintenance is metabolic nitrogen + endogenous nitrogen + cutaneous nitrogen.

Rose, 1950). In the early studies of Rose (1937, 1938), a diet of approximately 30 percent fat and with unrefined sources of B vitamins was used. In 1946, the fat content was reduced to 2–3 percent and crystalline vitamins were used (Bowman *et al.*, 1946). The problem introduced by altering the energy concentration of the diet was recognized by Rose's group (Wretlind and Rose, 1950), and they reexamined the requirements. Rama Rao *et al.* (1959) studied the essential amino acid requirements of the rat by supplementing 5 percent casein in a diet that contained 12 percent fat and later used 2 percent fat in a diet that contained amino acids and no protein (Rama Rao *et al.*, 1961). The amino acid requirements given in Table 6 are intended for use in a diet that contains 5 percent fat. Extrapolation of the requirements to diets of different caloric densities can probably be safely made by maintaining a constant amino-acid-to-calorie ratio and allowing for variations in amino acid digestibility (Guthneck *et al.*, 1953; Kornberg and Endicott, 1946; Lushbough *et al.*, 1957; Rogers and Harper, 1965; Schweigert and Guthneck, 1953, 1954).

Amino acid requirements are related to dietary protein concentration (Almquist, 1949; Becker *et al.*, 1957; Bressani and Mertz, 1958; Brinegar *et al.*, 1950; Grau, 1948). The requirements given in Table 6 are intended for use in a diet that contains 12 percent protein. In general, the requirement for an amino acid, expressed as percent of the diet, tends to increase as protein content increases but may remain constant or decrease slightly when expressed as percent of protein (Bressani and Mertz, 1958; Forbes *et al.*, 1955).

Requirements for leucine, isoleucine, threonine, valine, and phenylalanine were set considering the data reported from nutritional studies by Pick and Meade (1970), Rama Rao *et al.* (1959), Rose *et al.* (1949), Stockland and Meade (1970), and Stockland *et al.* (1971) and from the carcass analysis data of Williams *et al.* (1954). One-third to one-half of the phenylalanine requirement may be furnished as tyrosine (Rama Rao *et al.*, 1961; Stockland *et al.*, 1971). The value for tryptophan of 0.15 percent is intended for use in a diet that contains adequate amounts of niacin (Forbes and Rao, 1959; Hundley, 1947; Lushbough *et al.*, 1957; Osterling and Rose, 1952; Rama Rao *et al.*, 1961; Rose *et al.*, 1949; Salmon, 1954; Williams *et al.*, 1954; Young and Munro, 1973). The histidine requirement of 0.3 percent is based on data presented by Forbes and Yohe (1955), Harper (1959), Rama Rao *et al.* (1959), and Williams *et al.* (1954). The lysine requirement is 0.7 percent based on reports by Brookes *et al.* (1972), McLaughlan and Illman (1967), and Stockland *et al.* (1970), who found that lower levels were required than the 0.9 percent given in the previous edition of this publication (NRC, 1972). The requirement of 0.5 percent for isoleucine is a slight reduction from that given in 1972 (0.55 percent) and appears to be adequate according to results of Brookes *et al.* (1972) and the Minnesota group (Pick and Meade, 1971; Stockland and Meade, 1970; Stockland *et al.*, 1970, 1971). Amino acid requirements found by the Minnesota group are generally lower than given in Table 6 if expressed as percent of diet but tend to agree when given as percent of protein, because the group fed 10 percent protein.

The total sulfur amino acid requirement is 0.6 percent, of which one-half must be provided by methionine; the remainder may be provided by L-cystine (Hartsook and Mitchell, 1956; Schweigert and Guthneck, 1954; Shannon *et al.*, 1972; Wretlind and Rose, 1950). A lower requirement for sulfur amino acids fed as methionine (0.5 percent) has been reported by Rama Rao *et al.* (1961), Sowers *et al.* (1972), and Stockland *et al.* (1973). However, in view of the active role of methionine as a methyl donor, it is important to provide an allowance above the minimum needed for tissue synthesis (Aguilar *et al.*, 1974; Shannon *et al.*, 1972). The arginine requirement is set at 0.6 percent of the diet, based on reports by Hepburn and Bradley (1964), Ranhotra and Johnson (1965), and Rogers and Harper (1965), who showed a much higher requirement than that previously reported by Rose *et al.* (1949). The level of arginine needed for optimal results may be influenced by the amounts of glutamic acid and proline in the diet (Womack and Rose, 1947).

The difference between the total nitrogen requirement and the essential amino acid nitrogen requirement should be made up with mixtures of nonessential amino acids. Stucki and Harper (1962) reported that amino acid diets that contained both essential and nonessential amino acids gave better results in growing rats than diets that contained only essential amino acids or excess of nonessential amino acids. Ratios of nitrogen from essential and nonessential amino acids of 0.5 to 4.0 were satisfactory in diets that contained 9.4 to 15.0 percent protein. Breuer *et al.* (1963) found that purified diets which contained amino acids in the proportions recommended in the 1963 edition of this publication or the requirements stated by Rama Rao *et al.* (1959, 1961), would not support optimal growth. Studies by Hepburn and Bradley (1964), Ranhotra and Johnson (1965), and Rogers and Harper (1965) established that the diets were deficient in arginine. Breuer *et al.* (1964, 1966), Rogers and Harper (1965), and Newburg *et al.* (1975) showed that asparagine was required for maximal growth. Similarly, Hepburn and Bradley (1964) and Breuer *et al.* (1964) found that glutamic acid was necessary for maximal growth, and Breuer *et al.* (1964) and Adkins *et al.* (1966) showed that proline was required. The responses shown to these amino acids are presumed to be due to the inability of the rat to synthesize the quantities required in very rapid growth. However, as pointed out by Breuer et al. (1964) and Crosby and Cline (1973), rats appear to adapt to diets devoid of certain of the nonessential amino acids after a period of time and resume nearly maximal growth.

It is evident that specific requirements for the nonessential amino acids cannot be given because of the metabolic relationships among them. Therefore, the values given in Table 6 represent a pattern that has been used successfully in studies with purified diets. The value of 4 percent for glutamic acid is based on the data of Hepburn and Bradley (1964) and Breuer *et al.* (1964); that for asparagine is 0.4 percent, as found by Breuer *et al.* (1966) to be required for maximal growth. Proline is given at the level used by Adkins *et al.* (1966). To raise the total ration to 12 percent protein equivalent (percent N × 6.25), a mixture of alanine, glycine, and serine can be used.

Amino acid imbalances and antagonisms can result in increased requirements for individual amino acids, an area reviewed by Harper *et al.* (1970) and Harper and Rogers (1965). They concluded that the effects of imbalances and antagonisms on the requirement for maximum growth may be small if dietary protein concentration is adequate, but the effect in diets that contain suboptimal levels of protein may be considerable and suggest that the effect of imbalance is depression of feed intake.

Maintenance

Endogenous nitrogen excretion is related to basal metabolism (Brody, 1945; Mitchell, 1933, 1955). Thus, as with the maintenance energy requirement, the nitrogen requirement is properly expressed as a function of metabolic body size ($W_{kg}^{0.75}$) (Brody, 1945; Kleiber, 1975). In the 1972 edition (NRC, 1972) a median value of 200 mg N/$W_{kg}^{0.75}$ was calculated from the literature (Barnes *et al.*, 1946; Goettsch, 1951; Marshall and Womack, 1954; Womack *et al.*, 1953). The dietary protein requirement is 10.7 mg protein per gross kilocalorie, assuming a gross kilocalorie requirement of 117/$W_{kg}^{0.75}$ with 200 multiplied by 6.25.

The protein requirement (as ideal protein) is shown in Tables 4 and 6. Calculations for diets containing supplemented casein are shown in Table 4. A level of 7 percent is suggested for natural-ingredient diets. This value is in agreement with studies of Bricker and Mitchell (1947), in which milk or soy proteins were used instead of egg protein.

The essential amino acid requirements for maintenance of adult rats are based on reports by Benditt *et al.* (1950), Smith and Johnson (1967), and Said and Hegsted (1970). The data for each amino acid from these reports were averaged and are expressed on the basis of metabolic body size as follows (mg/$W_{kg}^{0.75}$): histidine, 23.5; isoleucine, 90.4; leucine, 53.1; lysine, 32.2; methionine, 67.2; phenylalanine, 54.5; threonine, 53.1; tryptophan, 15.6; and valine, 67.1. Assuming a basal energy requirement of 117 kcal/$W_{kg}^{0.75}$ for a 300-g rat, these data have been incorporated into Table 6 as a percentage of the diet. The requirements tend to be lower than those given in the previous edition (NRC, 1972), which were based entirely on the report of Benditt et al. (1950), but are considerably above the values reported by Nasset (1956). The determination of amino acid requirements for adult rats is difficult because of the flat dose–response curves that occur for many amino acids (Said and Hegsted, 1970; Smith and Johnson, 1967). Arbitrary decisions are therefore required in interpreting results, which helps account for the wide range in requirements reported by different investigators.

Gestation and Lactation

Adequate protein is essential for satisfactory reproduction and lactation in the rat. Nelson and Evans (1953) reported that 5 percent protein as unsupplemented casein was the minimal level that allowed reproduction to occur, while optimal performance occurred at 15–20 percent. The same investigators (1958) reported that 18 percent casein supported maximum growth in suckling young, but that 24 percent was required to provide for weight gain in the dam during lactation. Supplementary cystine was added to both diets. Sucrose was used as the source of carbohydrate, a factor that may have influenced results at lower levels of protein intake in their studies (Harper and Elvehjem, 1957). The lactating rat is sensitive to restriction of dietary intake (Peterson and Baumgardt, 1971b), which may occur because of the osmolar effect of sucrose (Harper and Spivey, 1958). Gander and Schultze (1955) reported good reproduction and lactation in rats fed 15–16 percent protein derived from a combination of casein, methionine, and mixed cereals. Similarly, Goettsch (1949) found 16.7 percent to be adequate in diets in which the true digestibility and biological value of the protein were 84.1 and 74.1, respectively. Sherman *et al.* (1949) reported that 20 percent protein was superior to 16 percent, when the diet contained milk products, wheat, and beef muscle.

It appears that, if data are corrected for digestibility and biological value, the net protein requirement for gestation and lactation as a percentage of the diet does not differ significantly from that for growth of weanling rats. The standard, therefore, has been set up to be identical with growth, and the data in Table 6 are so designated.

The amino acid requirements for gestation and lactation have been studied only briefly. A level of 0.11 percent tryptophan in diets that contained 1 or 2 percent nitrogen was found to be adequate to support normal pregnancy in rats (Lojkin, 1967). Nelson and Evans (1958) reported that the sulfur amino acid requirement for lactation was 1 percent of the diet, one-half of which could come from cystine. Greenstein *et al.* (1957) obtained excellent reproduction using a water-soluble purified diet supplemented with 3 percent corn oil, but weaning weights were below normal. The essential amino acid composition of the diets was nearly identical to the requirements for growth given by Rose (1938) and Rose *et al.* (1948), except that more arginine was present. Data are inadequate at this time to conclude that amino acid needs for gestation and lactation exceed the requirement for growth of young rats.

The requirement for protein for growth, gestation, and lactation, based on the research reviewed above, is 12 percent of air-dry diet as net protein (Table 4). For maintenance of adult rats, the requirement is 4.2 percent net protein. The requirements for amino acids for growth, gestation, and lactation in percent of diet are given in Table 6. A mixture of nonessential amino acids should be added to provide the equivalent protein required (Table 6).

Signs of Deficiency

Protein deficiency in growing rats results in growth reduction, anemia, hypoproteinemia, depletion of protein reserve, muscular wasting, emaciation, and, if sufficiently severe, death. In adults a loss of weight and body nitrogen occurs (Cannon, 1948), and chronic deficiency may lead to edema (Alexander and Sauberlich, 1957). Estrus becomes irregular and may cease; fetal resorptions occur, and the newborn are weak or dead. Similarly, reproductive capacity in the male is impaired (Goettsch, 1949). A lack of protein for pregnant and lactating rats may result in offspring that are stunted in growth (Hsueh *et al.*, 1967) with a reduction in DNA and RNA (Ahmad and Rahman, 1975; Zeman and Stanbrough, 1969). Low-protein diets also result in reduced food intake (Black *et al.*, 1950).

Removal of a single essential amino acid results in an immediate reduction in feed consumption, a situation that can return to normal within a day after replacement. Prolonged deficiency leads to a syndrome typical of protein deficiency (Cannon, 1948; Meister, 1957; Womack and Kade, 1944). A lack of an essential amino acid in the diet tends to be reflected by the concentration of the amino acid in the blood plasma (Kumta and Harper, 1962; Longenecker and Hause, 1959). A few specific signs characteristic of a lack of one amino acid have been reported: tryptophan—cataract formation, corneal vascularization, and alopecia (Cannon, 1948; Meister, 1957); lysine—dental caries, impaired bone calcification, blackened teeth, hunched stance, and ataxia (Bavetta and McClure, 1957; Cannon, 1948; Harris *et al.*, 1943; Kligler and Krehl, 1952; Likins *et al.*, 1957; Meister, 1957); methionine—fatty liver (Follis, 1958); arginine—increased excretion of urinary urea, citrate, and orotate (Milner *et al.*, 1974). The accumulation of a porphyrinlike pigment on the nose and paws has been observed in deficiencies of tryptophan, methionine, and histidine (Cole and Robson, 1951; Forbes and Vaughan, 1954), but this condition is also observed in other deficiency states.

MINERALS

The dietary requirements for minerals have been summarized in Table 6. A single value is given as the requirement for the growing rat, since data are not available on requirements at different stages of growth. For the same reason, maintenance values are not given.

In some reports, requirements were given as units per animal per day. The data have been converted to units per kilogram of diet on the basis of estimated feed intake of 15 g per day for growth, 20 g per day for gestation, and 30 g per day for lactation when feed intake data were not given.

One experiment has been reported (Bernhardt and Tomarelli, 1966) in which the 1963 recommendations for dietary minerals were tested for growth promotion in growing rats and were found to produce as good weight gain over a 21-day period as did 150 percent of the requirements. It thus appears that the requirements previously cited are correct for purified diets fed under conventional laboratory conditions. The 1972 edition cited the following changes in mineral requirements from the 1963 report: Calcium decreased from 0.6 to 0.5 percent, phosphorus from 0.5 to 0.4 percent, and iron increased from 25 to 35 mg/kg of the 90 percent dry-matter diet.

Calcium and Phosphorus

The requirements for calcium and phosphorus are approximately 0.5 and 0.4 percent of the diet for maximum calcification during growth. This is in general agreement with the data of Bernhart *et al.* (1969), Bethke *et al.* (1932), Chandler and Cragel (1962), and Evans and Ali (1967). Maximum weight gain may be attained with somewhat more than half of these amounts (Bernhart *et al.*, 1969). A ratio of calcium to phosphorus between 1.0 and 1.5 is recommended during the rapid growth period.

Maintenance requirements have not been established, but it is clear that a ratio of 2:1 of calcium to phosphorus is superior to 1:1 for prevention of osteoporosis (Draper *et al.*, 1972) (see also Vitamin D).

For reproduction, the earlier work of Cox and Imboden (1936) showed excellent performance at calcium and phosphorus levels of 0.49 percent. Their data also indicated that Ca:P ratios between 1:1 and 1.5:1.0 were optimal.

Signs of Deficiency Mild deficiencies of calcium and phosphorus produce few characteristic lesions other than impaired calcification and reduced weight gain. Severe deficiencies have not often been studied. Boelter and Greenberg (1941, 1943) fed 0.01 percent calcium diets to young rats for 8 weeks. The rats showed retardation of growth, decrease in food consumption, increase in basal metabolic rate, reduced activity and sensitivity, osteoporosis, rear leg paralysis, and internal hemorrhage. Males failed to mate, and females did not lactate properly. Day and McCollum (1939) fed 0.02 percent phosphorus diets to young rats. The animals survived up to 9 weeks and exhibited lethargy, pain, and cessation of bone growth. In numerous papers reviewed by Russell (1948) and McCoy (1949), reproduction was poor with either abnormal calcium-to-phosphorus ratios or low dietary content of each.

Chloride

There are few data on which to base a chloride requirement. St. John (1928) found that 0.05 percent chloride was adequate for growth. Voris and Thacker (1942) obtained a 25 percent growth reduction in a 10-week paired feeding comparison of 0.02 percent and 0.29 percent chloride. Picciano (1970) found no increase in weight gain of young rats fed 0.2 percent chloride compared to rats fed 0.05 percent. Miller (1926) reported that 5 mg/day was acceptable for reproduction and lactation. On the basis of these studies, 0.05 percent is set as the requirement.

Signs of Deficiency The rat tenaciously conserves its supply of tissue chloride by reducing drastically the urinary excretion within hours of consuming a diet deficient in the element. As a result, the signs of deficiency develop slowly. Rats fed a diet that contained 0.02 percent chloride (Pickens *et al.*, 1940) showed depression of appetite and reduction in body gain of nitrogen and energy; water consumption and heat production increased, while digestion and absorption remained normal. When a diet of 0.012 percent chloride was fed (Greenberg and Cuthbertson, 1942), there were no outward signs of deficiency except poor growth and reduction in blood chloride and chloride excretion. After consuming 0.005 percent chloride for 1 year, rats had poor growth and feed efficiency and abnormal renal glomeruli and tubules with fibrosis (Cuthbertson and Greenberg, 1945).

Chromium

For normal weight gain young rats required more than 0.17 mg/kg dietary chromium when care was taken to eliminate nondietary sources, but 2 mg/kg chromium in the drinking water permitted normal weight gains. (Schroeder, 1966). Normal gains were obtained with 0.3 mg/kg dietary chromium fed under usual dietary conditions (Staub *et al.*, 1969), and this concentration is used as the requirement.

Signs of Deficiency Diets that contained less than 0.17 mg/kg of chromium resulted in hyperglycemia and glycosuria similar to diabetes mellitus (Schroeder, 1966). Mature male weight and life span increased slightly when chromium was added to the drinking water at 5 mg/kg (Schroeder *et al.*, 1963). In rats fed a 10 percent soy diet, corneal opacity developed, which was reversed by addition of chromium (Roginski and Mertz, 1967). Chromium additions to low-chromium diets may lower serum cholesterol (Schroeder, 1969; Staub *et al.*, 1969).

Cobalt

Cobalt is apparently not required by the rat other than as a constituent of vitamin B_{12} (Underwood, 1962).

Copper (See Iron)

Fluoride

Under rigidly controlled experimental conditions, young rats gained maximally with 2.5, but not with 1.0, mg/kg fluoride in the diet (Schwarz and Milne, 1972). Under usual laboratory conditions no growth or other benefit was found from addition of fluoride to diets that contained less than 1 mg/kg (Maurer and Day, 1957), thus 1 mg/kg is set as the requirement.

Signs of Deficiency Fluoride deficiency was accompanied by bleached incisors and low weight gain. These abnormalities were partially prevented by fluoride supplements (Schwarz and Milne, 1972).

Iodine

The relatively few studies that have been conducted to determine the iodine requirement of the rat agree remarkably well that the requirement is between 0.100 and 0.200 mg/kg of diet (Halverson *et al.*, 1945; Levine *et al.*, 1933; Parker *et al.*, 1951; Remington and Remington, 1938). There appears to be no special requirement for reproduction; the studies of Parker *et al.* (1951) indicated that amounts between 0.100 and 0.225 mg/kg of diet were satisfactory. Kellerman (1934) reported that a natural-ingredient diet that contained 0.330 mg/kg supported excellent reproduction. The requirement for growth is set at 0.15 mg/kg.

Signs of Deficiency Iodine deficiency results in enlargement of the thyroid gland (Taylor and Poulson, 1956). Females deprived of iodine during pregnancy give birth to young with heavier thyroids than normal. Iodine deficiency inhibits reproduction (Feldman, 1960).

Iron and Copper

The iron requirement for growth and maintenance of maximal hemoglobin concentration derived from the data of McCall *et al.* (1962a) is about 35 mg/kg of diet. This is within the wide range of values in the older literature.

Reports of a requirement for copper have varied between 0.01 and 0.143 mg per rat per day (Hart *et al.*, 1928; Hundley, 1950; Keil and Nelson, 1931; Levine *et al.*, 1931, 1932; Mills, 1955; Mitchell and Miller, 1931; Pearson *et al.*, 1937; Rose *et al.*, 1934; Schultze *et al.*, 1934). The majority of these indicate that 5 mg/kg was a satisfactory dietary copper content. Larger amounts of copper may be needed to prevent achromotrichia. In one study the amount essential to prevent this condition was about 10 mg/kg (Mills and Murray, 1960). Mills (1955) suggested that the availability of copper from natural foodstuffs was superior to that of pure copper ion and indicated that earlier studies should be reevaluated. On the basis of these data, 5 mg/kg has been set as the requirement.

No separate requirements for iron and copper for reproduction have been established; 240 mg of iron per kilogram of diet supported satisfactory reproduction for three generations (McCall *et al.*, 1962a).

Signs of Deficiency Deficiency of either iron or copper results in hypochromic microcytic anemia (Smith and Medlicott, 1944; Underwood, 1962). In iron deficiency white incisor teeth, cardiomegaly, splenomegaly, and enlarged cecum develop (Cusack and Brown, 1965; McCall *et al.*, 1962b). Black-haired rats fed a copper- or iron-deficient diet developed achromotrichia (Cusack and Brown, 1965; Henderson *et al.*, 1942; Hundley, 1950; Keil and Nelson, 1931), which suggests an interrelation with pantothenic acid (Cusack and Brown, 1965; Singer and Davis, 1950). Copper deficiency increased blood cholesterol (Klevay, 1973).

When whole milk supplemented with manganese but not iron was fed to rats from weaning until they gave birth, they were anemic and produced anemic, nonviable young; copper-deficient dams were not anemic but gave birth to severely anemic edematous young with widespread subcutaneous hemorrhages (Odell *et al.*, 1961). Copper-deficient newborn rats exhibited behavioral changes that suggested a neurological disorder. They had a sixfold depression in brain copper concentration and deficiency of myelin formation in the cerebellum and brain stem (DiPaolo *et al.*, 1974).

Magnesium

McAleese and Forbes (1961) studied the influence of dietary magnesium on growth and magnesium concentration in bone and blood of the weanling rat. Dietary content of 100 mg/kg was adequate to support normal growth, but 350 to 425 mg of magnesium per kilogram of diet was required to maintain normal blood magnesium. Thus, 400 mg/kg appears to be the requirement for the growing rat.

Requirements suggested for the adult rat vary from a calculated figure of 50 mg/kg for replacement of endogenous losses (Smith and Field, 1963) to 2,500 mg/kg for normal bone histology (Clark and Belanger, 1967). Martindale and Heaton (1964) reported that 400 mg/kg may not be sufficient to maintain normal bone and serum magnesium in adult rats. Anderson (1970) found 400 mg/kg not adequate but 1,000 mg/kg adequate for rats fed diets high in calcium and phosphorus. Long-term maintenance of rats may require feeding diets that contain more than 400 mg/kg.

Requirements for reproduction have not been carefully evaluated, but 800 mg/kg provided as adequately for normal gestation and lactation as 1,900 mg/kg (Wang *et al.*, 1971).

Signs of Deficiency Deficiency of magnesium in the growing rat results in vasodilatation, hyperirritability, cardiac arrhythmias, spasticity, and fatal clonic convulsions. Vasodilatation occurs after about 1 week and may disappear and reappear spontaneously. Convulsions occur between 21 and 30 days (Ko *et al.*, 1962; Kunkel and Pearson, 1948; McCoy, 1949; Mickelsen *et al.*, 1955). Renal calcification is common (Forbes, 1964) and may be detected within 2 days after initiating a markedly deficient diet (Reeves and Forbes, 1972). Tufts and Greenberg (1938) reported that lactating females fed a deficient diet were bred successfully but did not suckle their young. Hurley *et al.* (1976a,b) reported that magnesium-deficient dams resorbed their fetuses or bore malformed pups and suggested that the malformations resulted from related zinc deficiency.

Manganese

The requirement of manganese for growth has not been adequately studied. Wachtel *et al.* (1943) reported reasonable gain with an intake of 0.05 mg per rat per day. Holtkamp and Hill (1950) obtained only slight improvement in growth when approximately 2 mg per rat per day was compared to 0.5 mg. Anderson and Parker (1955) showed that 50 mg/kg of diet was superior to 5 mg/kg. On the basis of these limited data, the requirement is set at 50 mg/kg of diet.

The requirement for manganese during reproduction was studied by Orent and McCollum (1932), Daniels and Everson (1935), and Richardson and Hogan (1940). From the values reported, a range between 0.35 and 1.2 mg per rat per day appears to be satisfactory for reproduction and lactation. Table 6 lists the requirement to be 50 mg/kg diet for growth, which will also support reproduction.

Signs of Deficiency An inadequate level of dietary manganese results in poor growth, defective mineralization of bone, reduced food consumption, and early mortality. Reproduction is impaired and is characterized by testicular degeneration in the male and defective ovulation in the female. If reproduction does occur, many of the young are ataxic because of defective otolith development (Hurley, 1968). Lactation is apparently not affected, since manganese-deficient mothers will suckle normal young satisfactorily. Hurley *et al.* (1961) have described skeletal abnormalities that occur in living young born to manganese-deficient dams.

Molybdenum

The fact that molybdenum is an integral part of xanthine oxidase, a hepatic enzyme, might lead one to deduce that it is a required nutrient (DeRenzo *et al.*, 1953; Richert and Westerfeld, 1953). However, feeding diets that contained approximately 0.020 mg/kg of molybdenum (approximately 0.2 µg per rat per day), or inhibition of xanthine oxidase with sodium tungstate, resulted in no abnormalities and did not impair growth of reproduction (Higgins *et al.*, 1956). On the basis of present information, a dietary requirement for molybdenum is not listed.

Phosphorus (See Calcium)

Potassium

Two studies of the requirement of potassium for growth (Grunert *et al.*, 1950; Kornberg and Endicott, 1946) suggested a level of 0.17–0.18 percent of the diet. Balance data of Heppel and Schmidt (1949) indicated that a concentration of 0.5 percent was adequate for lactation and 0.14 percent for gestation. Nelson and Evans (1961) confirmed that the requirement was between 0.5 and 0.6 percent for lactation. Recent studies have found that 0.36 percent is sufficient for growth and reproduction, which has been set as the requirement. The dietary concentration may be increased to 0.5 percent during lactation, but such an increase appears not to be required (American Institute of Nutrition, 1977).

Signs of Deficiency Insufficient potassium results in markedly reduced appetite and minimal growth. Animals become lethargic and comatose and may die within 3 weeks. They have an untidy appearance, cyanosis, short furlike hair, diarrhea, distended abdomens with ascites, and frequently hydrothorax. Pathological lesions are widespread (Kornberg and Endicott, 1946; Schrader *et al.*, 1937). Dietary concentration of 0.1 percent potassium resulted in symmetrical loss of hair along the back with a 50 percent reduction in hairs per follicular group (Robbins *et al.*, 1965).

Cardiac and renal lesions have been observed in potassium-deficient rats (Newberne, 1964). Initial noninflammatory degeneration of myocardial fibers was followed by necrosis and cellular infiltration. Renal lesions included cast formation in proximal convoluted tubules, sloughing of tubular epithelium in the medulla, and accumulation of hyaline droplets in the epithelium of the collecting tubules.

Selenium

A content of 0.040 mg selenium (as selenite) per kilogram of diet is necessary to prevent hepatic necrosis (Schwarz, 1958). Hafeman *et al.* (1974) found that 0.050 g/kg permitted maximum weight gain but that 0.100 mg/kg was required to maintain normal tissue concentration of glutathione peroxidase. The requirement is set at 0.100 mg/kg. Selenium is the most toxic of the essential minerals. Care should be taken to avoid dietary selenium concentrations above 1–2 mg/kg (Halverson, 1974).

Signs of Deficiency In the presence of adequate vitamin E, signs of severe selenium deficiency do not appear. This was demonstrated by Hurt *et al.* (1971), who fed a low-selenium, protein-free diet that contained amino acids with and without 0.5 mg/kg selenium for 20 weeks. The only differences between the groups were mildly depressed weight gain and markedly decreased tissue selenium in unsupplemented animals. At weaning, rat pups born to selenium-deficient mothers were nearly hairless and had atrophy of epidermis and epidermal appendages. No other gross or histological abnormalities appeared 6 weeks after weaning to a selenium-deficient diet. During this period, the pups gained 67 g compared to 93 g in pair-fed selenium-supplemented controls.

Sodium

The sodium requirement determined by Grunert *et al.* (1950) was 0.05 percent of the diet and was independent of potassium intake. This level is midway between the 0.03 percent found insufficient and 0.07 percent found sufficient by Miller (1923, 1926).

Data of Forbes (1966) indicated that 0.048 percent was inadequate for maximum weight gain of weanling rats over a 28-day period but that 0.22 percent gave maximal gains. Intermediate concentrations were not tested.

Pregnant females fed low-sodium diets (0.03 percent) ate less food and showed languor and debility, particularly during the last week of pregnancy; however, reproduction was not seriously impaired (Kirksey and Pike, 1962). Calculations from the older literature suggested a requirement during gestation and lactation of 0.13–0.5 percent of the diet (Kirksey and Pike, 1962; Miller, 1926; Nelson and Evans, 1961; Olson and St. John, 1925). Ganguli *et al.* (1969a,b) suggested a much reduced sodium requirement for gestation and lactation of 0.05 percent. On the basis of these data, the requirement is set at 0.05 percent.

Signs of Deficiency The classic sodium deficiency syndrome was described by Orent-Keiles *et al.* (1937). Rats fed a diet that contained 0.002 percent sodium, exhibited retarded growth, corneal lesions, and soft bones. Males became infertile after 2–3 months, and sexual maturity was delayed in females. Death ensued in 4–6 months. At a concentration of 0.007 percent sodium, Kahlenburg *et al.* (1937) noted reduced appetite; poor growth; increased heat production; and reduced stores of energy, fat, and protein.

Sulfur

Sulfur has not been classified as a required nutrient except as it is an integral part of the sulfur-containing amino acids and vitamins. However, Michells and Smith (1965) showed that dietary sulfate is readily incorporated into cartilage and will spare methionine for this purpose. It is suggested that 0.1 percent dietary sulfur be included when methionine is minimal. These data were supported by Bernhardt and Tomarelli (1966), who reported that a mineral mix that met the requirements reported in the first edition (NRC, 1963) of this report was improved by inclusion of 0.1 percent sulfate when fed in a low-protein (8.8 percent lactalbumin) diet. With adequate protein, no growth response was observed.

Jacob and Forbes (1969) obtained a slight but significant increase in 28-day weight gain of young rats fed a diet that contained 15 percent casein and methionine supplementation when the diet contained 0.035 percent sulfate sulfur rather than 0.004 percent. Smith (1973) reported that 0.02 percent dietary inorganic sulfate was optimum for adult male rats using as a criterion the reduced expiration of $^{14}\mathrm{CO}_2$ from a test dose of 1-^{14}C methionine. On the basis of these data, 0.03 percent sulfur as inorganic sulfate is set as the requirement.

Zinc

If rats are housed in galvanized cages, no more than 2–4 mg/kg of zinc is required (R. M. Forbes, personal communication). Rats maintained in a zinc-free environment and fed a diet that contains casein or egg white require 12 mg/kg for maximum weight gain (Forbes and Yohe, 1960; Pallauf and Kirchgessner, 1971). The requirement is higher (18 mg/kg) when isolated soybean protein is used. Petering *et al.* (1971) found 8 mg/kg in drinking water was required for maximum gain of young rats fed a purified diet that contained less than 2 mg/kg of zinc.

Signs of Deficiency An inadequate intake of zinc results in marked growth retardation and eventual growth failure, which are accompanied by mild anorexia, alopecia, thickening of the epidermis, loss of hair follicles, and hyperirritability (Underwood, 1962). Stirn *et al.* (1935) reported that the hair became soft, woolly, and light gray in color. The epidermal lesions are hyperkeratotic and may involve the esophagus as well as the skin.

When less than 2 mg/kg of zinc is fed to females, a severe disruption of the estrous cycle occurs, and in most cases no mating with normal males occurs (Hurley and Swenerton, 1966). A dietary concentration of less than 0.5 mg/kg zinc fed to growing males arrested spermatogenesis, resulted in atrophy of the germinal epithelium, and reduced growth of the pituitary and accessory sex organs. A similar finding was made by Luecke *et al.* (1968). If atrophy of the germinal epithelium and epididymides was severe, the damage was not reparable with zinc supplementation (Miller *et al.*, 1958). Growth of the accessory sex organs was promoted by gonadotropins in the absence of added dietary zinc (Miller *et al.*, 1958).

Teratogenic effects of zinc deficiency are extensive and include a high incidence of hydrocephaly and other central nervous system malformations, cleft palate, fused or missing digits, and urogenital abnormalities (Hurley and Swenerton, 1966; Warkany and Petering, 1972).

Other Elements

Evidence has accumulated recently to support inclusion of tin, silicon, vanadium, and nickel in a list of essential elements. To demonstrate a requirement for these elements, carefully designed diets deficient in them and an environment free of them were used. Environmental control included housing rats in plastic cages and isolators that receive only filtered air and feeding them water and diets devoid of the elements studied. Under such conditions, improved growth and health resulted from dietary additions of: tin, 1 mg/kg (Schwarz, 1974); silicon, less than 500 mg/kg (Schwarz, 1974); vanadium, 0.1 mg/kg (Schwarz, 1974); and nickel, 3 mg/kg (Nielsen and Ollerich, 1974).

FAT-SOLUBLE VITAMINS

Vitamin A

Vitamin A is absorbed in the small intestine as retinol, retinyl esters, or β-carotene. β-Carotene is transformed to retinol in the intestinal mucosa, and the retinol, irrespective of its source, is esterified to palmitate or stearate. The esters are transported to the liver and stored. When needed they are hydrolyzed to retinol and transported out of the liver to the target tissues in combination with a specific protein, retinol-binding protein.

Vitamin A is unusual in that it can be stored in the liver in large amounts. Mammals are born with very low liver stores. In consequence, the vitamin A requirement in weanling rats varies according to the criteria used, overt signs of deficiency (e.g., epithelial keratinization), or hepatic storage. Maximal blood levels (about 60 μg/100 ml) are reached when liver deposition is moderate (about 250 μg per gram of liver) (Muto *et al.*, 1972). The different criteria for vitamin A requirements for repletion of deficient animals are set out in Table 5 (Moore, 1957).

Vitamin A requirements are sensitive to other nutritional influences. For instance, protein-deficient diets, which deprive the animal of the ability to synthesize retinol-binding protein (Peterson *et al.*, 1974), resulted in low serum and tissue content of vitamin A; serum vitamin A concentration of rats fed a protein-free diet was reported to be nearly one-half normal (Mathews and Beaton, 1963). Liver stores of vitamin A were not affected by the level of protein intake when dietary vitamin A was normal, but vitamin A-depleted rats had greater liver stores on a low (4 percent) than a high (20 percent) protein intake (Mathews and Beaton, 1963). Vitamin A utilization, defined as rate of depletion of liver and kidney reserves, showed a direct linear relation with growth rate, which was changed by varying protein intake from 0 to 18 percent casein (Rechcigl *et al.*, 1962). Zinc deficiency has been reported to cause a decline in the mobilization of vitamin A out of the liver, but other studies have not confirmed the effect (Apgar, 1977; Smith *et al.*, 1973). Vitamin E deprivation resulted in increased depletion of liver stores of vitamin A (Moore, 1957).

TABLE 5 Vitamin A Repletion of Deficient Rats

Criteria for Repletion	Vitamin A Required (IU per rat per day)[a]
Growth restoration	2
Detectable hepatic storage of vitamin A	30
Full longevity	100
"Natural" storage[b]	100

[a] IU is defined as 0.300 μg retinol, or 0.344 μg retinyl acetate, 0.55 μg retinyl palmitate, or 0.6 μg β-carotene.

[b] Defined by Moore (1957a) as liver reserves equal to those found in wild animals.

Guilbert *et al.* (1940) demonstrated that the need for vitamin A was related to body weight rather than energy intake. This concept is consistent with the vitamin's activity in maintenance of integrity of epithelia, which quantitatively are directly correlated with body mass (Mitchell, 1950).

Early studies of vitamin A requirements (e.g., the often-quoted work of Sherman and Trupp, 1949), recommended an exceedingly high dietary concentration: 12,000 IU/kg of diet. The diets used were low in vitamin E and possibly other nutrients, and growth rates were low.

A precise recent study was made by Frier *et al.* (1975) of rats in a state of chronic vitamin A depletion. They were fed graded levels of vitamin A (as retinyl acetate) to determine that amount sufficient to prevent the three most sensitive indicators of vitamin A deficiency: decrease in growth rate, elevation of cerebrospinal fluid pressure, and squamous metaplasia of the nasolacrimal duct. The required amount was found to be 1,130 IU/kg of diet. In a similar experiment, Corey and Hayes (1972) tested graded levels of vitamin A in rats with acute vitamin A deficiency. Normal growth rate (5.6 g per day), normal serum vitamin A levels (43.7 μg/100 ml), and, the most sensitive indicator, normal cerebrospinal fluid pressure (30.9 mm H_2O) were achieved with a minimum of 2,560 IU/kg. Vitamin A was fed as retinyl acetate in beadlets. Maintenance of normal testicular development and spermatogenesis were achieved with about 1,000 IU retinyl acetate per kilogram of diet (Coward *et al.*, 1969).

The form in which vitamin A is fed is of importance because of its instability. Liver stores greater than 400 μg/g were achieved in rats fed the vitamin as retinyl acetate in gelatin-coated beadlets but not in rats fed the same amount added to the diet in petroleum ether (Bieri *et al.*, 1968).

In view of the instability of vitamin A and variability of the requirement under different environmental conditions, diets should contain more than the minimum requirement. The level recommended (J. G. Bieri, personal communication) for optimal growth, reproduction, tissue levels, and liver storage is 4,000 IU/kg for retinyl acetate or palmitate stabilized in gelatin-coated beadlets. If β-carotene is fed in gelatin beadlets, an amount of 4–6 mg/kg is recommended. The large excess is due to the fact that β-carotene is not absorbed as readily as vitamin A.

The route of administration, of course, affects the dose required for repletion. Rosso *et al.* (1975) reported that vitamin A-deficient rats could be maintained at the weight-plateau stage of deficiency with 1 IU/day per 100-g rat injected intravenously in aqueous emulsion. Vitamin A-deficient rats at the plateau stage regained growth rapidly when given intragastric administration of 10 IU retinyl acetate in oil per 100-g rat twice weekly or 3.6 IU intraperitoneally daily in aqueous emulsion (Sneider *et al.*, 1974).

Vitamin A occurs in various forms, of which the precursor (β-carotene) and the esters have been mentioned. From a practical standpoint, vitamin A acid (retinoic acid) is of importance. It is a normal metabolite of vitamin A (Ito *et al.*, 1974). It is rapidly excreted but is effective in maintaining growth and health of the rat, except for the processes of vision and reproduction. The effective dose is 2 mg/kg of diet (Lamb *et al.*, 1974).

Signs of Deficiency The signs of vitamin A deficiency can be divided into six categories. (1) Defect in vision. Since retinal is a necessary part of the visual pigment rhodopsin, deficiency of vitamin A leads to loss of vision through lack of visual pigment. (2) Bone defects. Vitamin A deficiency leads to disorganization of bone growth, retardation of skeletal growth, and failure of bone resorption during remodeling, giving rise, secondarily, to compression of nerves. (3) Increase in cerebrospinal fluid pressure. Defective fluid circulation may occur. (4) Reproductive failure. Cessation of spermatogenesis occurs in the male and resorption of the fetus in the female rat. (5) Epithelial metaplasia and keratinization. All epithelia are sensitive to vitamin A deficiency to varying degrees. In early vitamin A deficiency, goblet cell and mucous formation decline in the intestine; squamous metaplasia, followed by keratinization, takes place in the trachea. Keratinization of the urogenital tract and the corneal epithelium, combined with xerophthalmia and porphyrin deposits around the eyelids, and ultimately dissolution of the corneal stroma, takes place in severe vitamin A deficiency. (6) Growth failure. After 5–6 weeks of vitamin A deficiency, the weight of a weanling rat plateaus for about a week and then drops rapidly until the animal dies. Under germfree conditions, the rat can survive at the weight-plateau stage for several months (Rogers *et al.*, 1970).

A method for rapid, synchronous, vitamin A-deficiency production in groups of rats, using retinoic acid, has been described by Lamb *et al.* (1974).

Vitamin D

Vitamin D functions in serum calcium homeostasis. It is required, after conversion to active forms, together with parathyroid hormone, for mobilization of calcium from bone and for calcium and phosphorus absorption from the intestine (DeLuca, 1974). Dietary calcium and phosphorus levels strongly influence the effectiveness of vitamin D through their regulatory effects on an enzyme in the kidney that converts the precursor vitamin D metabolite, 25-hydroxycholecalciferol, to the active metabolite 1,25-dihydroxycholecalciferol. Serum calcium levels influence this enzyme via the parathyroid gland; serum phosphorus levels affect it directly. High dietary levels of both cause a decrease in conversion of the precursor to the active metabolite.

As stated by Boyle *et al.* (1971), "young rats absorb calcium more efficiently on a diet low in calcium (0.15 to 0.2%) than after a similar period of a diet high in calcium (0.8 to 1.25%)." At low dietary calcium concentration (e.g., 0.018 percent), the increased efficiency does not compensate and bone calcification cannot keep pace with growth, which results in inadequately calcified bones (Steenbock and Herting, 1955). The rat is especially sensitive to vitamin D deficiency and low dietary phosphorus in the presence of adequate dietary calcium (Steenbock and Herting, 1955). The above-mentioned renal enzyme functions adequately when serum calcium and phosphorus are normal (about 9 and 11 μg per 100 ml, respectively), but its activity increases to maximal values if either concentrations drop below these values (DeLuca, 1974).

The dietary requirements for calcium and phosphorus are approximately 0.5 and 0.4 percent, respectively, of the diet. When the diet contains these required amounts, the requirement for vitamin D is 1000 IU/kg of diet (H. F. DeLuca, personal communication) (1 IU is equivalent to 0.025 μg vitamin D_3 [cholecalciferol]).

Signs of Deficiency Vitamin D deficiency induces rickets. This disease is classically brought about in rats by a diet lacking vitamin D, adequate in calcium, and low in phosphorus. However, a low-calcium diet deficient in vitamin D has a more severe effect on growth rate and results in irritability, tetany and decreased bone calcification (Steenbock and Herting, 1955). Bones of rachitic rats show decreased or absent calcification with wide areas of uncalcified cartilage at the junction of diaphysis and epiphysis. Bone ash may be less than half normal. A full description of histological changes in bones of vitamin D-deficient rats is given by Jones (1971).

Vitamin E

Vitamin E has recently been found to function in concert with the enzyme glutathione peroxidase in preventing lipid peroxidation (Hoekstra, 1975). Therefore, those dietary factors (selenium, sulfur-containing amino acids) that had previously been thought to influence vitamin E requirement are now recognized rather to affect glutathione peroxidase activity and availability of glutathione, respectively. On the other hand, the dietary unsaturated fatty acids, which are highly susceptible to lipid peroxidation, influence vitamin E requirement. Older studies appeared to have established a fixed ratio between the amount of vitamin E required and the polyunsaturated fat content of the diet. More recently, Jager and Houtsmuller (1970) demonstrated that, at linoleic acid concentrations of about 3.5 percent, adequate weight gains were obtained and hemolysis was prevented with 13 mg D-α-tocopheryl acetate per kilogram of diet (equivalent to 17.6 mg DL-α-tocopheryl acetate) (1 mg DL-α-tocopheryl acetate is equivalent to 1 IU of vitamin E; 1 mg D-α-tocopheryl acetate is equivalent to 1.35 IU of vitamin E). However, increasing linoleic acid fourfold increased vitamin E requirement only by 40 percent.

The criterion of vitamin E requirement was prevention of spontaneous hemolysis. This criterion was extremely sensitive in the rat and correlated well with hyaline degeneration of gastrocnemius muscle; it was more sensitive than either decrease in weight gain or testicular degeneration (Jager, 1972). In a test of repletion of vitamin-deficient rats, Bieri (1972) showed that hemolysis was prevented after feeding 20 mg D-α-tocopheryl acetate per kilogram of diet. The dietary fat was a mixture of stripped corn oil and lard and provided 5.2 percent linoleic acid. This concentration also achieved stable levels of α-tocopherol in the tissues within 8 weeks following beginning of repletion.

The requirement, therefore, is 30 mg (30 IU) DL-α-tocopheryl acetate per kilogram of diet, in stabilized form (gelatin-coated), with a linoleic acid concentration of up to 5 percent, adequate dietary sulfur-containing amino acids, and selenium. Casein contains selenium, but the content can vary widely (Witting and Horwitt, 1964).

Signs of Deficiency The vitamin E-deficient rat has increased hemolysis, either spontaneous or brought about by treatment of the erythrocytes with oxidizing agents (dialuric acid). Other signs are: hyaline degeneration of skeletal muscle fibers with infiltration by histiocytes, interfibrillar fat cells, and increase in interstitial cells (Jager, 1972); accumulation of yellow pigment ("ceroid") in smooth muscle; irreversible degeneration of the seminiferous epithelium of the testis, which occurs by age 40–50 days; in the female induction of fetal abnormalities or intrauterine death and resorption; and hepatic degeneration, which develops at about 45–55 days in rats fed a vitamin E-deficient diet from weaning. Minimal microscopic changes occur in the liver 1–2 days before death, and massive necrosis occurs just before death.

Vitamin K

Vitamin K functions in the synthesis of prothrombin and three other blood-clotting factors: VII, IX, X. (DeLuca, 1974). In the absence of vitamin K, clotting time is prolonged. Dietary vitamin K requirement depends on the amount of vitamin K synthesized by the intestinal flora of the rat. Hence, variations in diet that influence intestinal synthesis leads to variable requirements. Further, the rat, which normally practices coprophagy, will need more vitamin K when restrained from coprophagy. The extent of coprophagy is dependent on the composition of the diet (Johnson *et al.*, 1960). Another consideration with regard to requirement is the vitamin K content of some dietary proteins. Unextracted casein may contain as much as 6 μg vitamin K$_1$ per kilogram (Matschiner and Doisy, 1965).

Adult male Wistar rats, permitted to practice coprophagy, and fed a 21 percent casein diet, require 50 μg vitamin K$_1$ (phylloquinone) per kilogram of diet for maintenance of normal prothrombin (Matschiner and Doisy, 1965). This last figure can be accepted as the requirement for vitamin K; it makes allowance for exceptionally low intestinal synthesis and a low contribution from casein.

Under unusual conditions, the requirement may be higher. Germfree rats required 200 μg/kg (Wostmann *et al.*, 1963) and 100 μg/kg when coprophagy was prevented (Mameesh and Johnson, 1960). Menadione is considered to be approximately one-tenth as active as vitamin K, and, therefore if used, should be added to diets in 10 times the amount suggested in Table 6.

Repletion experiments were described by Matschiner and Taggart (1968). Intracardiac injection of 12 μg of vitamin K per kilogram of body weight gave complete recovery within 18 hours. Knauer *et al.* (1975) achieved rapid repletion by feeding a soy protein diet that contained 140 μg of vitamin K$_1$ per kilogram to vitamin K-deficient Sprague-Dawley rats. Prothrombin content was restored to normal within 3 days.

Signs of Deficiency Vitamin K deficiency depresses the ability of the rat liver to convert a precursor of prothrombin to prothrombin and hence leads to lowered serum prothrombin levels (to 10 percent of normal), which results in prolonged blood-clotting time and hemorrhage. Wostmann *et al.* (1963) studied germfree rats with vitamin K deficiency and described splenomegaly and decreased hemoglobin and hematocrit, in addition to lowered prothrombin; presumably the anemia was caused by bleeding.

Vitamin K deficiency is produced within 2 weeks in rats fed a soy protein diet free of vitamin K (Matschiner and Taggart, 1968). The process is accelerated by feeding sulfonamides (Almquist, 1971), or preventing coprophagy (Mameesh and Johnson, 1959). Female rats are more resistant to deficiency than male rats (Johnson *et al.*, 1960). Hypervitaminosis A can lead to vitamin K deficiency (Matschiner and Doisy, 1962). This effect is thought to be due to an interference by vitamin A with vitamin K absorption (Matschiner *et al.*, 1967).

WATER-SOLUBLE VITAMINS

The water-soluble vitamins function as coenzymes in a wide range of enzymatic reactions. They are absorbed primarily in the upper small intestine, with the exceptions of vitamin B$_{12}$ and possibly riboflavin, which are absorbed in the ileum (Chanarin, 1971; Middleton and Grice, 1964). They are synthesized by intestinal bacteria and are available to rats through coprophagy if not directly through absorption (Hotzel and Barnes, 1966).

Ascorbic Acid

Rats do not require a dietary source of ascorbic acid; however, certain B vitamins may be spared when ascorbic acid is fed. It has been suggested that ascorbic acid increases fecal vitamin content, which is restored to the rat via coprophagy. Five percent ascorbic acid in the diet supported normal weight gain in thiamin-deficient rats and increased the fecal content of thiamin (Murdock *et al.*, 1974; Scott and Griffith, 1957).

Biotin

Biotin is not required under standard laboratory conditions, as it is supplied in adequate levels by intestinal bacterial

TABLE 6 Nutrient Requirements of Rats

Nutrient		Concentration in a Diet[a] Growth, Gestation, or Lactation	Maintenance
Protein (as ideal protein)	%	12.00	4.20
Fat[b]	%	5.00	5.00
Digestible Energy	kcal/kg	3,800.00	3,800.00
L-Amino Acids			
Arginine	%	0.60	—
Asparagine	%	0.40	—
Glutamic acid	%	4.00	—
Histidine	%	0.30	0.08
Isoleucine	%	0.50	0.31
Leucine	%	0.75	0.18
Lysine	%	0.70	0.11
Methionine	%	0.60[c]	0.23
Phenylalanine-tyrosine	%	0.80[d]	0.18
Proline	%	0.40	—
Threonine	%	0.50	0.18
Tryptophan	%	0.15	0.05
Valine	%	0.60	0.23
Nonessential[e]	%	0.59	0.48
Minerals			
Calcium	%	0.50	
Chloride	%	0.05	
Magnesium	%	0.04	
Phosphorus	%	0.40	
Potassium	%	0.36	
Sodium	%	0.05	
Sulfur	%	0.03	
Chromium	mg/kg	0.30	
Copper	mg/kg	5.00	
Fluoride	mg/kg	1.00	
Iodine	mg/kg	0.15	
Iron	mg/kg	35.00	
Manganese	mg/kg	50.00	
Selenium	mg/kg	0.10	
Zinc	mg/kg	12.00	
Vitamins			
A[f]	IU/kg	4,000.00	
D[f]	IU/kg	1,000.00	
E[f]	IU/kg	30.00	
K_1	µg/kg	50.00	
Choline	mg/kg	1,000.00	
Folic acid	mg/kg	1.00	
Niacin	mg/kg	20.00	
Pantothenate (calcium)	mg/kg	8.00	
Riboflavin	mg/kg	3.00	
Thiamin	mg/kg	4.00	
Vitamin B_6	mg/kg	6.00	
Vitamin B_{12}	µg/kg	50.00	

synthesis. It is the coenzyme for acetyl coenzyme A (acetyl-CoA) carboxylase, which is responsible for the synthesis of malonyl-CoA in *de novo* synthesis of long-chain fatty acids and for several other transcarboxylases and is required for purine synthesis (Baker and Frank, 1968; Numa *et al.*, 1970).

Signs of Deficiency Induction of biotin deficiency in the rat requires feeding raw egg white, which binds and prevents absorption of the vitamin. Signs of deficiency are exfoliative dermatitis, alopecia, achromotrichia, and an abnormal, spastic gait. In rats fed 10 percent raw egg white, oral administration of 2 µg of biotin per rat per day prevented development of deficiency. That amount would be supplied by a dietary content of 0.15 mg/kg (Nielsen and Elvehjem, 1941). In rats fed biotin-deficient diets that contained 20 percent sprayed egg white for 10 days, biotin-dependent enzymes ranged from 20 to 80 percent of normal. Control rats given 200 µg of biotin parenterally once a week had normal levels of enzymes, and deficient animals given a single injection of 200 µg restored their enzyme levels to normal within 4 to 24 hours (Chiang and Mistry, 1974). That level of biotin corresponds approximately to 2 mg/kg of diet. Hepatic gluconeogenesis was decreased in deficient rats and was restored to normal 3 hours after injection of 200 µg of biotin (Sandoval and Sols, 1974).

Choline

Choline is a component of lecithin in soluble and membrane phospholipids and of the neurotransmitter, acetylcholine. It is required by the rat, but it can be replaced in diets formulated from amino acids and in other purified components by methionine (Newberne *et al.*, 1969). Interactions among choline, methionine, folate, and vitamin B_{12} are complex and have been elucidated in part through their lipotropic action, i.e., their ability to maintain normal hepatic lipid metabolism and prevent accumulation of triglyceride in liver cells, their effects on hepatic uptake and storage of folates, and their effects on the enzymes for methionine synthesis and degradation (Best *et al.*, 1954, 1969; Finkelstein *et al.*, 1971; Griffith and Nye, 1954; Gyorgy *et al.*, 1967; Hale and Schaefer, 1951; MacDonald *et al.*, 1965; Newberne *et al.*, 1969; Thenen and Stokstad, 1973; Vitale and Hegsted, 1969). The transmethylation of homocysteine to methionine by methyl groups derived from choline or other methyl donors requires folate coenzymes and vitamin B_{12} and is presumably central to the interaction of these compounds.

Notes to Table 6

[a] Adequate to support growth, gestation, and lactation; based on 90 percent dry matter.

[b] Linoleic acid, 0.6 percent, is required.

[c] One-third to one-half can be supplied by L-cystine.

[d] One-third to one-half can be supplied by L-tyrosine.

[e] Mixture of glycine, L-alanine, and L-serine.

[f] Vitamin A, 1 IU = 0.300 µg retinol, 0.344 µg retinyl acetate, 0.550 µg retinyl palmitate.
Vitamin D, 1 IU = 0.025 µg ergocalciferol.
Vitamin E, 1 IU = 1 mg DL-α-tocopheryl acetate.

The dietary requirement for choline is influenced by the lipid content of the diet, the chain length and degree of saturation of dietary lipids, and the total caloric content of the diet (Best *et al.*, 1954; Patek *et al.*, 1966; Salmon and Newberne, 1962; Zaki *et al.*, 1965). In adequate diets, which provide 4 to 4.5 kcal/g, the requirement is approximately 0.1 percent. However, addition of choline up to 0.4 percent may be required for diets that contain 20 percent or more fat or are low in methionine.

Signs of Deficiency Lipotrope-deficient diets are deficient in choline, folate, vitamin B_{12}, and methionine. A dietary lipid content of 15–30 percent by weight is fed to increase severity of the deficiency (Best *et al.*, 1954; Rogers and Newberne, 1973). Male rats are more susceptible to the deficiency than female rats (Patek *et al.*, 1969). Droplets of neutral triglyceride and abnormalities of intracellular membranes are demonstrable in liver cells of weanling male rats within 24 hours after they have ingested a lipotrope-deficient diet. Long-term deficiency leads first to fatty liver, in which triglycerides compose as much as 50 percent of the total wet weight of the liver and the liver cells are markedly distended with fat vacuoles, and then to cirrhosis, in which there is proliferation of fibrovascular tissue, vascular shunting, and hepatic failure (Hartroft, 1963; Rogers and MacDonald, 1965; Zaki *et al.*, 1963).

Deficiency of choline in young rats has marked effects also on both the kidney and cardiovascular system. If male rats are fed a choline-deficient diet at weaning, 50 to 90 percent die within 10 days to 2 weeks of hemorrhagic renal necrosis. They may develop myocardial necrosis and atheromatous changes in arteries (Monserrat *et al.*, 1974; Salmon and Newberne, 1962).

Protection against renal damage is given by diets that contain approximately half the amount required to prevent fatty liver. Partial supplementation also prevents or retards development of cirrhosis despite the presence of increased fat in the liver. Marginal deficiencies of lipotropic agents may depress hepatic drug metabolism and immunological functions (Newberne and Gebhardt, 1973; Rogers and Newberne, 1971). Marginal deficiency in pregnant rats induces persistent metabolic and immunologic abnormalities in the offspring (Williams *et al.*, 1975).

Folates

Folates are conjugated with one or more glutamic acid residues; the major storage form in rat liver is the pentaglutamate (Houlihan and Scott, 1972; Kutzbach *et al.*, 1969). The folate coenzymes function in transfer of 1-carbon units in synthesis of thymidine, purines, methionine, and many other compounds. Measurement of tissue content of the several forms of folate and of urinary excretion of formiminoglutamic acid (FIGLU), a histidine catabolite that cannot be normally metabolized in folate deficiency, is utilized to assess folate nutrition (Baker and Frank, 1968). The dietary requirement in rats fed an adequate diet is met by intestinal synthesis, but diets inadequate in choline, methionine, and vitamin B_{12} may induce deficiency of folate through the extensive interactions of these compounds (Thenen and Stokstad, 1973; Vitale and Hegsted, 1969). Dietary concentrations of 0.5–10.0 mg/kg have been used and 1.0 mg/kg is recommended.

Signs of Deficiency Induction of deficiency requires prolonged feeding of diets deficient in lipotropes (see Choline) or containing either antibiotics to decrease the supply of folate from intestinal bacteria or folate antagonists. Decreased growth rate, leukopenia, anemia, and FIGLU excretion are induced, but megaloblastic bone marrow or intestinal cells are only occasionally reported. Specific defects are induced in thiamin absorption, cell-mediated immunity, and protein synthesis (Hautvast and Barnes, 1974; Howard *et al.*, 1974; Vitale and Hegsted, 1969; Williams *et al.*, 1975).

Inositol

Inositol is not required by rats under conventional laboratory conditions, but Burton and Wells (1976) reported a requirement in lactating rats fed antibacterial drugs.

Niacin

Niacin as an amide combined with adenine, D-ribose, and phosphate forms the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which function with dehydrogenases in intermediary metabolism. It is synthesized from tryptophan, and, therefore, pure deficiency of niacin does not occur. From studies of the interaction between tryptophan and niacin, it was calculated that 33–40 mg of tryptophan yield 1 mg of niacin and that the niacin requirement of the rat was between 13 and 30 mg/kg of diet when tryptophan was at a minimal level of 0.15 percent, the amount supplied by 11 percent casein (Hankes *et al.*, 1948; Harris and Kodicek, 1950). Weanling male Sprague-Dawley rats fed a semisynthetic diet that contained either 18 mg/kg or 180 mg/kg of niacin showed no difference in weight gain over a period of 23 days from weaning (Kao and Forbes, 1973). Diets that contained 20 to 90 mg/kg all yielded approximately the same liver stores of the vitamin (Gaudin-Harding *et al.*, 1971). A dietary content of 20 mg/kg is adequate even at minimally adequate tryptophan intake.

Signs of Deficiency Force-feeding of diets deficient in both niacin and tryptophan induces development of behavioral changes, convulsions, diarrhea, rough hair coat, and alopecia (Hundley, 1954). The changes can be reversed by feeding niacin or tryptophan, but deficiencies induced by feeding corn may be complicated by development of amino acid imbalances (Gopalan and Rao, 1972). The neurotransmitter, serotonin, which is synthesized from tryptophan, was decreased in deficient rats, and full restoration required complete protein supplementation rather than tryptophan supplementation alone (Fernstrom and Wurtman, 1971).

Pantothenic Acid

Pantothenic acid functions primarily as a constituent of coenzyme A in synthesis of lipids and steroids and in acetylation reactions. The dietary requirement for growth and reproduction and, specifically, for maintenance of acetylation reactions is 8 mg/kg of calcium pantothenate (Barboriak *et al.*, 1957a,b).

Signs of Deficiency Pantothenic acid deficiency induces achromotrichia, exfoliative dermatitis, oral hyperkeratosis, necrosis, and ulceration of the gastrointestinal tract. Focal or generalized hemorrhagic necrosis of the adrenals may occur, and death results after 4–6 weeks of deficiency (Ralli and Dumm, 1953). Deficient rats had impaired antibody synthesis, decreased serum globulins, and decreased antibody-forming cells in response to antigen. Restoration of antibody synthesis was achieved by parenteral administration of calcium pantothenate beginning 9 days before antigen injection. Normal antibody synthesis was maintained in rats given an oral supplement of 300 μg/day, which corresponds to a diet content of 20 mg/kg and is probably excessive (Lederer *et al.*, 1975; Roy and Axelrod, 1971).

Riboflavin

Riboflavin is the precursor of the flavin coenzymes and is stored in the liver primarily as flavin adenine dinucleotide (FAD) (Rivlin, 1970). The coenzymes function with many oxidation-reduction enzymes, e.g., cytochrome C reductase, xanthine oxidase, and diaphorase. They are required, as is vitamin B_6, for conversion of tryptophan to nicotinic acid. Riboflavin is required for normal metabolism of vitamin B_6 and folate coenzymes (Baker and Frank, 1968; Rivlin, 1970; Tamburro *et al.*, 1971). The requirement is influenced by the dietary content of carbohydrate. Starch increases intestinal synthesis of the vitamin and decreases the required level in the diet; sucrose does the reverse. Maximum hepatic storage was found in rats fed 40 μg/day (equivalent to 2.7 mg/kg of diet) and maximum growth at 30 μg/day (equivalent to 2 mg/kg of diet) (Bessey *et al.*, 1958). A dietary content of 0.9 or 1.2 mg/kg gave hepatic storage in rats equivalent to that given by 15.6 or 23.0 mg/kg (Gaudin-Harding *et al.*, 1971). Studies to determine the absolute requirement for riboflavin suggested that it may be as low as 3.2 μg per rat per day, the equivalent of 0.2 mg/kg of diet, but the dietary level recommended was 17 μg per rat per day, or about 1.2 mg/kg of diet (Anonymous, 1972).

Offspring of dams fed 1 mg/kg riboflavin in the diet did not grow normally and had decreased body and brain weight and brain desoxyribonucleic acid (DNA) content compared to offspring of rats fed 8 mg/kg during gestation and lactation and themselves fed 2.7 mg/kg after weaning. Correction of defects in the deficient young was achieved by feeding their dams 2.7 mg/kg in the diet during lactation, but not by supplementation of the diet of the offspring after weaning (Fordyce and Driskell, 1975). Four mg/kg in the diet of pregnant rats gave growth and hepatic riboflavin stores equivalent to 100 mg/kg (Schumacher *et al.*, 1965).

The dietary requirement based on growth and hepatic stores is 2-3 mg/kg, but at least 3 mg/kg is required for normal reproduction and an increase to 4 mg/kg may be advisable during gestation. The requirement can be expressed on a caloric basis as 0.6–0.8 mg/1,000 kcal.

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Signs of Deficiency The classical signs of riboflavin deficiency are dermatitis, alopecia, weakness, and decreased growth. Corneal vascularization and ulceration, cataract formation, anemia, and myelin degeneration may occur (Horwitt, 1954). Deficient rats may have fatty liver, abnormal hepatocyte mitochondria, and metabolic abnormalities of hepatocytes. The complex metabolic effects of riboflavin deficiency have been reviewed and summarized as: (1) a decrease in flavoproteins involved in cellular oxidations; (2) increased protein turnover and an increased pool of free amino acids, which result in increased amounts of enzymes associated with amino acid metabolism, particularly enzymes of gluconeogenesis; and (3) a large decrease in mitochondrial respiration and adenosine triphosphate (ATP) synthesis (Garthoff *et al.*, 1973). Reproductive performance is decreased in both males and females; offspring of deficient females may have congenital anomalies.

In rats fed purified diets deficient in riboflavin, red cell and hepatic riboflavin levels were decreased by 4 weeks and continued to decrease thereafter. Red cell glutathione reductase was significantly decreased by 4 weeks, and the hepatic content of the enzyme was significantly decreased at 5 weeks (Bamji and Sharada, 1972). Rats deficient in niacin had depressed hepatic riboflavin content despite adequate dietary levels of the vitamin, and rats deficient in riboflavin had depressed hepatic folate stores despite adequate or even increased intake of folate (Tamburro *et al.*, 1971).

Thiamin

Thiamin is the precursor of thiamin pyrophosphate, which is the storage form, and the coenzyme for oxidative decarboxylation and other oxidative reactions.

The requirement for thiamin in the diet of the rat is dependent in part on quantity and source of dietary calories, is increased by increasing carbohydrate, and may be decreased by increasing dietary fat (Scott and Griffith, 1957). Recent studies have not supported this effect of fat (Murdock *et al.*, 1974).

Male weanling rats fed a diet that contained either 1.25 mg/kg or 12.5 mg/kg of thiamin did not differ in food efficiency ratio, but rats fed the higher level grew faster (Mackerer *et al.*, 1973). There was no significant difference in growth of rats fed either 5 or 50 mg thiamin per kilogram of diet (Itokawa and Fujiwara, 1973). In rats fed a 12 percent casein, 1.2 percent fat diet that contained either 0.15 or 1.6 mg/kg of thiamin or a 32 percent casein, 47 percent fat diet that contained 0.10 or 2.3 mg/kg of thiamin, stores of thiamin in the liver were increased by the two higher dietary concentrations (Gaudin-Harding *et al.*, 1971).

Pregnant rats were fed diets that contained either 4 mg/kg or 100 mg/kg of thiamin. Hepatic stores of thiamin were increased at weaning in the offspring of dams fed the higher concentration, but there was no significant effect on growth (Schumacher *et al.*, 1965). The requirement is 4 mg/kg.

Signs of Deficiency Thiamin deficiency can be induced readily and produces abnormalities of the central and peripheral nervous system and the heart and results in poor reproductive performance. Anorexia and weight loss are prominent; blood pyruvate may be elevated.

In a study of the effect of progressive thiamin deficiency on biochemical parameters in cardiac muscle and other tissues, it was found that thiamin-deficient rats developed encephalopathy and cardiac hypertrophy after 5 weeks. Deficient rats had ataxia, impaired "righting" reflex, and drowsiness, which were reversed by injection of thiamin hydrochloride. Brain thiamin content was significantly decreased. Cardiac weight was increased by an average of 18 percent, but no abnormality of the heart was found by light or electron microscopy. Cardiac and renal ATP and pyruvate carboxylase were significantly reduced at 4 weeks and returned to normal within 24 to 72 hours after administration of thiamin (McCandless *et al.*, 1970; Schenker *et al.*, 1969).

The enzymatic activity of transketolase in blood and tissues of thiamin-deficient animals correlates with thiamin status, and it may or may not be restored *in vitro* by the addition of thiamin pyrophosphate (TPP). This may depend on the duration of the deficiency and the resultant instability of the apoenzyme (Bamji and Sharada, 1972; Brin, 1966; Pearson, 1967; Warnock, 1970). Folate-deficient rats had decreased absorption of low doses of thiamin but large doses were absorbed normally (Howard *et al.*, 1974). Folate-deficient rats fed 22 mg/kg in the diet had significant depletion of blood and liver but not brain levels of thiamin (Thompson *et al.*, 1972).

Vitamin B_6

The vitamin B_6 compounds (pyridoxine, pyridoxal, and pyridoxamine) function as coenzymes for amino acid decarboxylases, racemases, transaminases, and other enzymes in amino acid, glycogen, and fatty acid metabolism (Baker and Frank, 1968). The coenzymes are formed by phosphorylation of the aldehyde and amine; nearly 50 percent of pyridoxal phosphate in the body is stored as coenzyme for muscle glycogen phosphorylase (Anonymous, 1975; Chen and Marlatt, 1975). Studies of the dietary requirement have been based on enzyme activities, body weight gain, tissue stores of pyridoxal phosphate, or reproductive performance. When male weanling rats were fed 1, 2, 4, or 8 mg of vitamin B_6 per kilogram of diet, growth was the same in all groups, but liver, serum, and red cell glutamic–pyruvic transaminase (GPT) was maintained only at dietary concentrations of 4 mg/kg and above and could be stimulated by the addition of B_6 *in vitro* even in tissues taken from rats fed 8 mg/kg (Chen and Marlatt, 1975). Red cell transaminase activity is a more sensitive indicator than hepatic transaminase activity or maximal hepatic storage of vitamin B_6 and indicates a requirement of 6–7 mg/kg (Beaton and Cheney, 1965).

Vitamin B_6 is required in the diet of pregnant rats for normal development of their offspring. Deficient offspring have retarded renal differentiation, abnormalities of cerebral lipids, and increased tissue and urinary levels of cystathionine (DiPaolo *et al.*, 1974; Kurtz *et al.*, 1972; Pang and Kirksey, 1974). Maternal weight gain and body and brain weight of offspring were normal when the diet contained 3 mg/kg of pyridoxine and slightly but not significantly lower at 2 mg/kg; 1 mg/kg was clearly inadequate. There was no significant difference between offspring of dams fed 3 mg/kg and those fed 6 mg/kg (Driskell *et al.*, 1973). In another study in pregnant female rats, the dietary concentration of vitamin B_6 required for maximum tissue vitamin content was between 9.6 and 19.2 mg/kg, but enzyme levels were normal at 2.4 mg/kg (Kirksey *et al.*, 1975).

Weanling females fed diets that contained 1.2 to 19.6 mg/kg of B_6 through breeding, gestation, and lactation bore offspring of normal weight at intakes of 2.4 mg/kg and above; brain content of the vitamin, protein, and cerebrosides was significantly decreased at 1.2 and 2.4 mg/kg, and brain protein content continued to increase up to 19.6 mg/kg. Milk content of pyridoxine was decreased at maternal intakes below 4.8 mg/kg, as was red cell transaminase activity. The minimum adequate intake was 4.8 mg/kg, except when judged on the basis of brain protein (Moon and Kirksey, 1973; Pang and Kirksey, 1974).

The dietary requirement for growth and reproduction is therefore at least 5 mg/kg and for maintenance of normal transaminase activity is approximately 7 mg/kg.

Signs of Deficiency Rats fed diets deficient in vitamin B_6 develop symmetrical scaling dermatitis on the tail, paws, face, and ears; microcytic anemia; hyperexcitability; and convulsions (Sherman, 1954). Reproductive performance of both females and males is decreased; deficient production of insulin may occur (Huber *et al.*, 1964). In rats fed a 70 percent casein diet deficient in B_6, urinary urea decreased from 80 to 50 percent of total nitrogen; blood urea decreased; and urinary excretion of free ammonia decreased, while excretion of free amino acids increased. Cystathionine and citrulline were excreted in large amounts by deficient rats. The activity of hepatic serine and threonine dehydratases and cystathionase decreased (Okada and Suzuki, 1974).

Vitamin B_{12}

In mammals, vitamin B_{12} is required as a coenzyme for the transmethylation of homocysteine to methionine, utilizing 5-methyl-tetrahydrofolic acid, and in the conversion of methylmalonyl-CoA to succinyl-CoA (Weissbach and Taylor, 1970). A role of vitamin B_{12} in catabolism of methionine has been described in rats fed toxic levels of methionine. The toxicity was blocked by addition of 150 μg B_{12}/kg of diet. Lower concentrations were not tested (Areshkina *et al.*, 1973). The required level of supplementation of vitamin B_{12} in the diet of the rat varies with dietary content of choline, methionine, and folic acid. Induction of isolated vitamin B_{12} deficiency in the rat, as well as in other experimental animals, is achieved with difficulty and generally does not reproduce the signs of human vitamin B_{12} deficiency—megaloblastic blood cells and neurological lesions.

The requirement for vitamin B_{12} is met adequately by a dietary content of 50 μg/kg.

Signs of Deficiency Deficiency can be induced in rats fed vegetable rather than animal protein (which contains vitamin B_{12}). Female rats fed a diet that contained soybean protein supplemented with methionine and choline, but not vitamin B_{12}, grew normally or at a slightly decreased rate and bred and littered normally; but the average weight of their offspring was decreased, and 10 percent of the litters had hydrocephalic members. Hepatic content of vitamin B_{12} was markedly decreased in both mothers and offspring. Deletion of choline from the diet increased the incidence of congenital abnormalities in the neonates. Supplementation of the diet with 50 μg/kg of vitamin B_{12} supported normal growth in the mothers and prevented development of hydrocephalic offspring (Woodard and Newberne, 1966). Germfree rats fed soybean protein aborted, bore short-lived pups, or cannibalized their pups. The germfree condition apparently enhanced B_{12} deficiency (Valencia and Sacquet, 1968). Vitamin B_{12} deficiency has been induced by diets containing unheated soybean flour, but amino acid deficiencies occur also and there is evidence of toxicity; therefore, it has been suggested that its use for the study of vitamin B_{12} deficiency is not justified (Edelstein and Guggenheim, 1971; Williams and Spray, 1973).

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NUTRIENT REQUIREMENTS OF THE MOUSE

Mice have been used extensively as laboratory animals, but research on nutrient requirements for growth, reproduction, lactation, or maintenance of this species has received little attention. As a result, most estimates of nutrient requirements for mice must be based on (1) data accumulated many years ago involving mouse strains or diet ingredients that are no longer available or cannot be identified, (2) experimental results derived from studies not directed toward establishment of requirements, (3) estimated nutrient consumption by mice fed diets producing "acceptable performance," or (4) the assumption that mouse requirements are similar to those of the rat.

An additional problem in attempting to define nutritional requirements for mice is the numerous genetic strains available to investigators using this species. Strain differences affect growth rates (Poiley, 1972), and nutrient requirements for growth, reproduction, and maintenance (Fenton, 1957; Fenton and Carr, 1951; Fenton and Marsh, 1956; Goodrick, 1973). Genetic factors may also affect the degree of response to a nutritional deficiency (Hurley and Bell, 1974). The Institute of Laboratory Animal Resources (NRC, 1975) maintains a registry of sources for the various stocks and strains of mice. The genetic characteristics of outbred mouse stocks and various strains of inbred and mutant mice have been listed (Green, 1975; NIH, 1974), and tables showing considerable differences in the growth rate among stocks and strains of mice have been published (Poiley, 1972).

Table 7 provides a summary of growth in 26 inbred mouse strains demonstrating some observed growth rates. Averages for 2 outbred stocks and 10 hybrid lines were within the same ranges. The original data show great variability among strains. An indication of maximum possible weight gain in a nonselected, hybrid line (Swiss X CF1) was the report by John and Bell (1976) of a gain of 18 g in 14 days (1.3 g/day). Canolty and Koong (1976) observed a gain of 1.3 g/day for 21 days in an inbred line selected for rapid postweaning gain over 41 generations.

A major aspect of the study of nutrient requirements is the question: Requirements for what? Rapid postweaning growth that leads to maximum body size at maturity is the most readily available measure of dietary adequacy. Some investigators have assumed that diets adequate for such growth would also be adequate for maximum reproductive rates, as well as for long-term maintenance. However, Knapka *et al.* (1977) found that diets that produced maximum postweaning growth did not necessarily support maximum rates of reproduction. Since the mouse achieves one-third of its total growth during the suckling period, as compared with one-fifth for the rat, lactation imposes a heavier nutritional burden on the dam, which may affect some dietary requirements more than others. Dubos *et al.* (1968) found that a casein-starch diet calculated to contain 0.05 percent magnesium was adequate for growth of mice, but caused sudden death of some females during lactation. An additional 0.02 percent magnesium prevented the syndrome. This indicates that the demand for magnesium rose more sharply than the demand for total diet. Reproductive efficiency is affected by strain differences even more than is growth, so that standards of performance for each strain must be obtained from the general literature.

The other assumption, that a diet that promotes maximum growth and/or maximum reproduction is the best for long-term maintenance, is being increasingly questioned (Ross and Bras, 1975; Ross *et al.*, 1976), because unnecessarily high consumption of some nutrients (or of total diet) may be life-shortening. The mechanisms of this life-shortening effect are being intensively studied. The meaning of the

TABLE 7 Average Body Weight and Range of Averages for 26 Inbred Mouse Strains (Poiley, 1972)

	Females		Males	
Age (days)	Average (g)	Range of Averages (g)	Average (g)	Range of Averages (g)
21	10.2	7.5–14.8	11.0	7.7–15.8
28	14.2	9.9–18.6	15.7	11.3–20.5
42	18.6	13.1–25.8	21.0	14.4–28.7
56	21.5	15.1–28.9	24.1	16.1–30.8
112	28.1	21.1–37.6	30.2	23.2–35.7

term "adequacy" must therefore be expanded to indicate a range of nutrient intakes between minimal and harmfully excessive; the range will vary at different stages of the life cycle. Nutrition investigators have generally focused on nutrient requirements as *minimum* dietary concentrations, except for a few nutrients with known toxic potential, like vitamins A and D. For lifetime studies, however, *maximal* dietary concentrations of other nutrients may eventually have to be defined. Investigations are now being carried out into the effects of diet on longevity of mice.

Some aspects of dietary adequacy still to be investigated, especially with regard to longevity, involve the role of diet in promoting a beneficial gut flora (Floch, 1967; Pesti and Gordon, 1973) and in providing optimal stimulation for immune function, peristalsis, and possibly other functions. New categories of dietary requirements (e.g., fiber) can be anticipated. These may prove beneficial for longevity even if they reduce the availability of some nutrient(s).

Maintaining mice in germfree, gnotobiotic, or specific pathogen-free (SPF) environments, where the kinds and number of intestinal microorganisms are minimal, may have a significant effect on the required dietary concentration of various nutrients. Luckey *et al.* (1974) fed a sterilized diet marginal in several vitamins and observed decreased reproduction in germfree as compared to conventionally reared mice. Observations made in the mouse colonies maintained by the Small Animal Section at the National Institutes of Health indicated a dramatic improvement in reproduction of some mouse strains when moved from a conventional to an SPF environment, while other strains reproduced readily only in a conventional environment (J. J. Knapka, unpublished data).

Estimation of quantitative dietary requirements for B vitamins has been complicated by the fact that B vitamins are both synthesized and used up by the intestinal microflora (Mickelsen, 1956) and that the products of bacterial synthesis become available in varying amounts by way of coprophagy (Daft *et al.*, 1963). Even the list of qualitative B vitamin requirements may be expanded by conditions that minimize the contribution of intestinal microbial synthesis, such as maintenance under germfree, gnotobiotic, or some very restricted SPF environments; feeding of antibiotics or of highly absorbable diets; and prevention of coprophagy. A requirement for myoinositol by mice will therefore be discussed in this chapter, even though it is not required under conventional conditions of intestinal microbial synthesis.

A further complication is that B vitamins are among the most labile nutrients in ground mixed diets, being reduced by storage, pasteurization, and sterilization. Zimmerman and Wostmann (1963) studied the effect of autoclaving for 25 minutes at 121°C on the destruction of B vitamins in several air-dried diets. Losses were as follows: thiamin, 75–90 percent; B_6, 17–25 percent; pantothenic acid, 33–47 percent; riboflavin, 5–12 percent. The addition of more water to the diet before autoclaving reduced the losses substantially.

The increasing use of SPF, gnotobiotic, and germfree mice requires that substantially higher levels of B vitamins in the original diet may be necessary to compensate for the vitamins lost in diet treatment and those customarily produced by the intestinal microflora. Therefore, a number of commercial suppliers now offer autoclavable, B vitamin fortified versions of standard diets. Germfree mouse colonies at Lobund Laboratory fed one such autoclavable diet (Kellogg and Wostmann, 1969) (Table 8), have maintained normal growth and reproduction through many generations, even during

TABLE 8 Examples of Formulas for Satisfactory Natural-Ingredient Diets for Mice

	Amount Added per Kilogram of Diet[a]		
	Conventional		Autoclavable
Ingredient	Knapka *et al.*, 1974	Bell, 1972	Kellogg and Wostmann, 1969
Ground wheat, g	230	400	—
Wheat middlings, g	100	—	—
Ground corn, g	245	—	590
Corn gluten meal, g	30	—	—
Ground barley, g	—	333	—
Stabilized lard, g	—	20	—
Corn oil, g	—	—	30
Soybean oil, g	25	—	—
Dehydrated alfalfa meal, g	40	50	35
Soybean meal, g	120	75	300
Brewer's yeast, dried, g	20	20	—
Nonfat dry milk solids, g	50	—	—
Fish meal, g	100	50	—
Lysine, g	—	—	5
Methionine, g	—	—	5
Dried molasses, g	15	30	—
Steamed bone meal, g	—	13	—
Dicalcium phosphate, g	12.5	—	10
Iodized salt, g	7	5	—
Salt, g	5	—	10
Ground limestone, g	5	—	—
Calcium carbonate, g	—	3	5
BHT, g[b]	—	—	0.125
Cobalt, mg	0.44	—	0.66
Copper, mg	4.4	—	2.2
Iodine, mg	1.5	—	1.3
Iron, mg	132	—	22
Manganese, mg	66	—	66
Zinc, mg	17.6	25	15
Vitamin A, IU	6,060	1,500	26,400
Vitamin D, IU	5,070	150	1,000
α-Tocopheryl acetate, mg	22	—	220
Vitamin K, mg	2.9	—	88
Choline chloride, mg	570	—	1,980
Folic acid, mg	2.4	—	11
Niacin, mg	33	—	66
Pantothenate, mg	19.8	—	286
Riboflavin, mg	3.7	—	31
Thiamin, mg	11.0	—	66
Pyridoxine, mg	1.9	—	22
Vitamin B_{12}, μg	4.4	—	4.4

[a] The amount listed is the quantity added during formulation of the diet, not the total content of a given vitamin or mineral in the diet. The trace minerals are added in a variety of salt forms; see references for details.

[b] Butylated hydroxytoluene; an antioxidant.

TABLE 9 Examples of Formulas for Satisfactory Purified Diets for Mice, in Percent

Ingredient	AIN-76[a]	Hurley[b]	Bell[c]
Casein, 85 percent protein commercial	20.0	—	—
Casein, purified high nitrogen[d]	—	30.0	—
Casein, vitamin free	—	—	21.2
DL-Methionine	0.3	—	0.15
Cornstarch	15.0	—	27.5
Sucrose	50.0	—	7.6
Glucose	—	54.5	25.6
Cellulose	5.0	—	5.5
Fat	5.0[e]	8.0[e]	7.6[f]
Mineral mix	3.5[g]	6.0[h]	4.5[i]
Vitamin mix	*1.0*[j]	1.5[k]	0.5[l]
Choline bitartrate	0.2	—	—

[a] American Institute of Nutrition (1977). This diet is intended for growth and maintenance during the first year of life. Investigators should be aware that diets high in sucrose can be cariogenic. The diet has been found to be satisfactory for reproduction and lactation in both rats and mice. If used for deficiency studies, modifications will be necessary.

[b] Erway *et al.*, 1970; Bell and Hurley, 1973; Hurley and Bell, 1974.

[c] National Research Council (1972).

[d] Not vitamin free.

[e] Corn oil.

[f] Equal parts lard and vegetable shortening, stabilized with 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline.

[g] Percent of mineral mix: $CaHPO_4$, 50.0; NaCl, 7.4; K citrate$\cdot H_2O$, 22.0; K_2SO_4, 5.2; MgO, 2.4; manganous CO_3, 0.35; ferric citrate, 0.60; $ZnCO_3$, 0.16; $CuCO_3$, 0.03; KIO_3, 0.001; $Na_2SeO_3\cdot 5H_2O$, 0.001; $CrK(SO_4)_2\cdot 12H_2O$, 0.055; sucrose, powdered, 11.8.

The total dietary calcium and phosphorus, respectively, with various protein sources will be approximately (mg per 100 g of diet): crude casein, 656 and 620; purified casein, 520 and 560; isolated soy protein, 558 and 552.

[h] Percent of mineral mix: $CaCO_3$, 30.0; K_2HPO_4, 32.1; NaCl, 16.8; $MgSO_4\cdot 7H_2O$, 12.5; $CaHPO_4$, 6.0; $FeSO_4\cdot 7H_2O$, 2.5; KI, 0.08; $ZnCO_3$, 0.025; $CuSO_4\cdot 5H_2O$, 0.030; $MnSO_4\cdot H_2O$, 0.23.

[i] Percent of mineral mix: NaCl (iodized), 4.54; $CaHPO_4$, 77.20; $KHCO_3$, 15.33; MgO, 2.03; $MnSO_4\cdot H_2O$, 0.34; $FeSO_4\cdot 2H_2O$, 0.34; $CuSO_4\cdot 5H_2O$, 0.11; and ZnO, 0.11.

[j] Per kg of vitamin mixture (g): thiamin$\cdot$HCl, 0.6; riboflavin, 0.6; pyridoxine$\cdot$HCl, 0.7; niacin, 3.0; Ca pantothenate, 1.6; folic acid, 0.2; biotin, 0.020; vitamin B_{12}, 1 mg; vitamin A, 400,000 IU; vitamin E, 5,000 IU; vitamin D_3, 2.5 mg; vitamin K, 5.0 mg; sucrose, powdered, to make 1,000 g.

[k] Per kg of vitamin mixture (g): folic acid, 0.03; biotin, 0.125; vitamins A and D_3 (each 325,000 IU/g) 0.23; *p*-aminobenzoic acid, 0.5; riboflavin, 0.5; menadione, 1.25; nicotinic acid, 1.5; pyridoxine, 1.5; thiamin$\cdot$HCl, 1.5; vitamin B_{12} (1 mg/g), 1.5; vitamin A (325,000 IU/g), 2.1; Ca pantothenate, 2.5; ascorbic acid, 5.0; vitamin E (125,000 IU/g), 21.4; inositol, 25.0; choline chloride, 50.0 dextrose to make 1,000 g.

[l] Per kg of diet: vitamin E (IU), 140; menadione (mg), 200; choline (mg), 4,000; thiamin (mg), 5; riboflavin (mg), 10; niacin (mg), 50; pyridoxine (mg), 5; pantothenic acid (mg), 50; folic acid (mg), 2.5; biotin (mg), 1; vitamin A, stabilized (IU), 5,000; vitamin D (IU), 1,250; vitamin B_{12} (μg), 250; inositol (mg), 2; methionine, DL- (g), 1.5.

occasional periods when germfree rats fed the same diet showed reproductive decline which responded to supplementation of the drinking water with the B vitamin complex (B. A. Teah, private communication).

Germfree environments have been found to affect the availability of some dietary minerals. Concentrations of calcium that were not excessive for conventional mice have caused soft tissue calcification in germfree mice (Wostmann, 1975). By contrast, germfree rabbits absorbed inorganic iron less well than did conventionally reared rabbits (Wostmann, 1975).

Germfree and gnotobiotic mice also require diets that cause as little distention of the cecum as possible under germfree conditions, since such distention can affect growth, survival, reproduction, and metabolic rate.

The exact factors that affect cecal size have not been determined, but they appear to have a physiological rather than nutritional effect (Gordon and Pesti, 1971). Diets have been developed empirically to reduce cecal distention and thus provide normal growth and reproduction in mice (Wostmann, 1975) and above average longevity (Gordon *et al.*, 1966).

Numerous natural-ingredient, purified, and chemically defined diets have been formulated for mouse colonies maintained for both experimental and production purposes. Table 8 contains examples of natural-ingredient diet formulations that have produced acceptable growth and reproduction in mice reared under conventional and SPF environments. The diet of Knapka *et al.* (1974) has been recommended as the standard natural-ingredient reference diet for research by the American Institute of Nutrition. Examples of purified diet formulations are presented in Table 9. The diet designated as AIN-76 has been developed and evaluated as a standard purified reference diet by a committee of the American Institute of Nutrition (1977). It contains regular casein (85 percent), whereas the purified diet of Bell (1972) contained vitamin-free casein as the protein source. The AIN-76 diet is designed to be an easily reproducible diet rather than a highly purified basal diet for the study of nutritional deficiencies. Formulations for chemically defined mouse diets have been published by Pleasants *et al.* (1970, 1973).

Estimated nutrient requirements for mice are presented in Table 10. In general, they are estimated for conventional mice. Users of germfree or gnotobiotic mice should consult Wostmann (1975) for discussion of increased qualitative and quantitative requirements under these environmental conditions. As noted above, SPF colonies sometimes display deficiency signs when fed diets adequate for conventional mice. Table 11 provides the calculated nutrient concentrations of the representative natural-ingredient and purified diets given in Tables 8 and 9. In general, the nutrient concentrations in natural-ingredient diets are in considerable excess of the estimated requirements. The diet of Knapka *et al.* (1974) is comparable in nutrient concentrations to some of the most generally used commercial diets. Producers of laboratory mice have had extensive experience with commercial diets, and many experimental mouse colonies also are maintained on them.

Comparison of the requirements listed in Table 10 and the concentrations in Table 11 suggests that more efficient production and perhaps more valid biomedical research may be achieved by feeding diets that contain lower concentrations of various nutrients. As the later discussions of individual nutrients will show, considerably more research

TABLE 10 Estimated Nutrient Requirements of Mice[a] Adequate for Both Growth and Reproduction, Except Where Noted

Nutrient	Unit	Requirement[b]	Comments
Linoleic acid	%	0.3	Based on rat requirement
Protein (growth)	%	12.5	John and Bell, 1976
Protein (reproduction)	%	18.0	Knapka *et al.*, 1977
L-Amino acids			For growth (John and Bell, 1976); not tested for reproduction
Arginine	%	0.3	
Histidine	%	0.2	
Isoleucine	%	0.4	
Leucine	%	0.7	
Lysine	%	0.4	
Methionine	%	0.5	Partly replaceable by L-cystine
Phenylalanine	%	0.4	Partly replaceable by L-tyrosine
Threonine	%	0.4	
Tryptophan	%	0.1	
Valine	%	0.5	
Minerals[c]			
Calcium	%	0.4	Est. from content of adequate diets
Chloride			Required; no quantitative data
Magnesium	%	0.05	For growth; may be low for reprod.
Phosphorus	%	0.4	Est. from content of adequate diets
Potassium	%	0.2	Bell and Erfle, 1958
Sodium			Required; no quantitative data
Chromium	mg/kg	2.0	Est. from content of adequate diets
Copper	mg/kg	4.5	Lowest in diets adq. for growth
Fluoride			Status uncertain for mice
Iodine	mg/kg	0.25	Est. from Abbassi and McKenzie, 1970
Iron	mg/kg	25.0	For growth; 120 mg/kg is lowest content yet tested for reprod.
Manganese	mg/kg	45.0	Est. from content of adequate diets
Selenium			Required; no quantitative data
Vanadium			No data for mice; may be needed in ultraclean environments
Zinc	mg/kg	30.0	Est. from content of adequate diets
Vitamins			
A	IU/kg	500.0	IU = 0.3 μg retinol equivalent
D	IU/kg	150.0	IU = 0.025 μg cholecalciferol equiv.
E	IU/kg	20.0	IU = 1 mg DL-α-tocopheryl acetate
K_1 equivalent	mg/kg	3.0	Est. from content of adequate diets
Biotin	mg/kg	0.2	Est. from content of adequate diets
Choline	mg/kg	600.0	NRC, 1972
Folacin	mg/kg	0.5	Est. from content of adequate diets
Inositol (myo-)			Bacterial synthesis usually adequate
Niacin	mg/kg	10.0	NRC, 1972
Pantothenate (Ca)	mg/kg	10.0	NRC, 1972
Riboflavin	mg/kg	7.0	NRC, 1972
Thiamin	mg/kg	5.0	NRC, 1972
Vitamin B_6	mg/kg	1.0	NRC, 1972
Vitamin B_{12}	mg/kg	0.01	Est. from content of adequate diets

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[a] These are estimated minimal requirements for conventional mice. The concentrations of certain nutrients will have to exceed the concentrations given when conditions tend to increase an animal's requirement (e.g., germfree status) or decrease the stability or availability of some nutrients.

[b] The concentrations listed are based on weights of ingredients containing an average of 10 percent moisture.

[c] Other minerals have recently been suggested as necessary for rats (e.g., nickel, tin, and silicon) in ultraclean environments. They have not been studied in mice.

TABLE 11 Nutrient Composition of Diets Which Appear Adequate for Mice

		Amounts in Adequate Diets[a]				
Nutrient	Unit	Unrefined[b] Natural Ingredient	AIN-76[c]	Hurley[d]	Bell[e]	Pleasants[f]
Protein	%	24	17 +	30	21.2	14.3
Fat, total, including linoleate	%	5.3	5	8	7.6	5
Linoleic acid	%					2.3
Carbohydrate	%		65	54.5	60.7	73.2
Fiber	%	3.5	5	0	5.5	0
L-Amino acids						
Arginine	%	1.3				0.63
Asparagine	%					0.80
Histidine	%	0.54				0.57
Isoleucine	%	1.13				0.74
Leucine	%	1.96				1.37
Lysine	%	1.28				1.37
Methionine	%	0.53				0.74
Cystine	%	0.37				
Phenylalanine	%	1.12				0.57
Tyrosine	%					0.57
Threonine	%	0.96				0.57
Tryptophan	%	0.37				0.29
Valine	%	1.24				0.57
Nonessential	%					5.5
Minerals						
Calcium	%	1.23	0.52	0.81	1.02	0.57
Chloride	%		0.16		0.12	1.03
Magnesium	%	0.18	0.05	0.073	0.055	0.142
Phosphorus	%	0.99	0.4	0.42	0.79	0.57
Potassium	%	0.85	0.36	0.89	0.27	0.40
Sodium	%	0.36	0.1	0.39	0.08	0.38
Sulfur	%				0.007	0.0023
Chromium	mg/kg		2	1.9		4
Cobalt	mg/kg	0.7				0.2
Copper	mg/kg	16.1	6	4.5	12.6	12.9
Fluoride	mg/kg					2.3
Iodine	mg/kg	1.9	0.2	36		3.8
Iron	mg/kg	255	35	299	50	47.6
Manganese	mg/kg	104	54	50	50	95.2
Molybdenum	mg/kg					1.55
Selenium	mg/kg		0.1			0.076
Vanadium	mg/kg					0.25
Zinc	mg/kg	50.3	30	31	40	38.0
Vitamins						
A	IU/kg	15,000	4,000	1,100	5,000	1,730
D	IU/kg	5,000	1,000	1,100	1,250	171
E	IU/kg	37	50	32	140	1,514
K_1 equiv.	mg/kg	3	0.05	18	200	10.7
Biotin	mg/kg	0.2	0.2	0.2	1	1
Choline	mg/kg	2,009	1,000[g]	750	4,000	2,375
Folacin	mg/kg	4	2.0	0.45	2.5	1.43
Inositol (myo-)	mg/kg				2	238
Niacin	mg/kg	82	30.0	22.5	50	35.6
Ca pantothenate	mg/kg	21	16.0	37.5	50	47.5
Riboflavin	mg/kg	8	6.0	7.5	10	7.1
Thiamin	mg/kg	17	6.0	22.5	5	4.8
Vitamin B_6	mg/kg	10	7.0	22.5	5	6.0
Vitamin B_{12}	mg/kg	0.03	0.01	0.023	0.25	0.58

Continued

to determine the nutrient requirements of mice will be required to resolve this issue.

ENERGY

Troelsen and Bell (1963) found that mice consumed an average of 3.5 g of diet daily during 14 days postweaning. A variety of diets that supported good growth were found to be providing intakes of 14.5 kcal per day per mouse of metabolizable energy. Canolty and Koong (1976) reported that a line of mice selected for rapid postweaning growth ate 5 g of diet (18 kcal of metabolizable energy) per day from 21 to 42 days of age, whereas mice of the same strain not selected for rapid growth consumed 3.8 g of diet (14 kcal of metabolizable energy) per day. Nevertheless, the energy requirement for maintenance was exactly the same for the two strains: 176 kcal per unit of metabolic body size ($W^{0.75}$) per day.

The dietary concentrations of the major nutrients are important in terms of their relationship to each other, as well as their absolute amounts. Bosshardt *et al.* (1948) demonstrated that fat and carbohydrate were equal in their protein-sparing effect for growing mice.

The maximum content of crude fiber or cellulose compatible with normal or maximal growth rates depends on the nature of the fibrous material, since it may affect palatability, digestion, lactation, intestinal microbial biosynthesis, and intake of other nutrients (Bell, 1960; Dalton, 1965). Most mouse diets contain approximately 5 percent crude fiber.

PROTEIN AND AMINO ACIDS

Bing *et al.* (1932) fed Bagg albino mice purified diets that provided 15.2, 23.2, or 49.7 percent of total calories from casein and observed postweaning growth similar to that of mice fed a natural-ingredient diet that contained approximately 19 percent crude protein. When casein provided 7.8 percent of the calories, the mice grew at a slightly subnormal rate. Korsrud (1966) reported that weight gain of CF1 mice approached maximum when the diet contained 11.3 percent protein (14 percent of total calories) from egg protein, or 11.9 percent protein (13.8 percent of total calories) from fish meal. Goettsch (1960), feeding purified diets, found that 13.6 percent casein was the minimal dietary protein concentration that supported acceptable growth, reproduction, and lactation in Swiss STM mice.

John and Bell (1976) obtained maximal growth of a fast-growing hybrid line when the diet contained 12.5 percent protein (as casein and amino acids), which has been accepted as the growth requirement (Table 10). Hoag and Dickie (1960) observed no differences in the weaning weight of C57BL/6J or DBA/2J mice weaned from dams that had been fed commercial rations containing 17 or 25 percent crude protein.

Natural-ingredient diets with crude protein concentrations ranging from 20 to 25 percent are fed to many mouse colonies maintained for experimentation and reproduction. Knapka *et al.* (1974) reported acceptable reproductive performance in BALB/cAnN, C57BL/6N, and N:NIH(S) mice maintained on a natural-ingredient diet containing 24 percent crude protein. In a more recent report, Knapka *et al.* (1977) using the BALB/cAnN, C57BL/6N, C3H/HeN, and DBA/2N strains found that 18 percent dietary protein was at least as effective for reproduction as 24 percent protein when crude fat concentrations were 4 and 8 percent, respectively. Pleasants *et al.* (1973) observed greater lactation efficiency in germfree C3H mice fed a diet containing 14 percent free amino acids than in one containing 24 percent free amino acids. The C3H strain is known to have a low-protein requirement relative to calories (Fenton and Carr, 1951). Bruce and Parkes (1949) reported only slight differences in reproductive performance of mice fed natural-ingredient diets that contained 13.6, 15.0, or 18.4 percent digestible protein. In contrast, Hoag and Dickie (1962) reported weaning a larger percent of C57BL/6J mice when the dietary crude protein concentration was increased from 17 percent to 20 percent in a natural-ingredient diet containing 11 percent fat; reproductive performance of AKR/J mice was at a maximum when the diet contained 20 percent crude protein, and lower at 22 and 24 percent.

The foregoing data indicate considerable variation among mouse strains in the protein concentration needed for efficient reproduction, and also a variation due to differences in the fat content of the diet. The traditional approach to recommendation of a protein content for mouse diets has been to start with the minimal requirement of the most demanding strains and add several percent to allow for variations in protein quality. This approach accounts for the concentration of about 24 percent found in most commercial diets. However, the data of Knapka *et al.* (1977) indicated that an 18 percent protein diet was slightly superior to a 24 percent protein diet for reproduction. Therefore, this report recommends an 18 percent dietary protein content for reproduction, which is adequate for most strains and not excessive for any (Table 10).

John and Bell (1976) determined the individual amino acid requirements for maximal growth of a hybrid mouse

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Notes to Table 11

[a] The amounts given are based on weights of ingredients in equilibrium with normal room humidity.

[b] Knapka *et al.*, 1974.

[c] American Institute of Nutrition, 1977.

[d] Erway *et al.*, 1970; Bell and Hurley, 1973; Hurley and Bell, 1974.

[e] Bell, 1972.

[f] Pleasants *et al.*, 1973.

[g] Choline content of 2,000 mg/kg choline bitartrate.

(Swiss X CF1) gaining 1.3 g per day. These are listed in Table 10. Theuer (1971) determined the amino acid requirements of C57BL/6J mice toward the end of the growth period (from thirty-ninth to sixtieth day of age), when maximum gain was 3 g in 3 weeks. These requirements, which are about half those given by John and Bell (1976), are probably very close to maintenance requirements. Amino acid requirements for reproduction have not been reported.

Leveille *et al.* (1961) demonstrated a requirement for 0.47 percent of sulfur-containing amino acids in diets that contained 2.5 percent nitrogen (15.6 percent protein) and 0.26 percent in diets with 1.5 percent nitrogen (9.4 percent protein). Bauer and Berg (1943a) showed that the mouse could synthesize cystine when methionine was provided. Beard (1926) found that mice could not utilize taurine as a source of sulfur for amino acid synthesis. Bauer and Berg (1943b) found that D- and L-methionine and D- and L-phenylalanine all promoted growth, but only the L-isomers of valine, leucine, isoleucine, and threonine promoted growth. Omission of arginine did not reduce the growth rate. More recently, Milner *et al.* (1975) reported a temporary reduction in growth rate of mice when arginine was removed from a purified diet. Totter and Berg (1939) observed that growth of mice was retarded when D-tryptophan and D-histidine were fed instead of the L-isomers and that D-lysine failed to promote growth of mice when it was added to diets deficient in L-lysine. Celander and Berg (1953) confirmed that mice could not utilize D-histidine or D-tryptophan. However, Harding-Gaudin (1961) found that some mice could utilize D-tryptophan. The requirements listed in Table 10 are given in terms of the L isomers. If DL mixtures are used, the requirements need to be recalculated on the basis of the above information.

FAT

A requirement for fat as such, not merely as a source of essential fatty acids, was postulated for rats by Lassen and Bacon (1949). Bosshardt *et al.* (1950) found, however, that the mouse required no more than 0.5 percent dietary fat for growth if the diet contained adequate vitamin B_{12}. They reviewed reports of other nutrient deficiencies in which fat played an ameliorative role but was not needed when the deficiencies of the other nutrients were corrected.

If the metabolizable energy concentration in a diet is below that required for growth or reproduction, then substituting fat (9 kcal/g) for carbohydrate (4 kcal/g) provides a means of increasing the metabolizable energy concentration (caloric density). Caloric density requirements for the mouse have not been determined. Most mouse diets contain about 5 percent fat and about 5 percent fiber, yielding a metabolizable energy content of about 4 kcal/g. Increasing the fat level above the usual 5 percent will ordinarily lower the daily intake of other nutrients. If any nutrient is already minimal, increasing the dietary fat content could lead to a deficiency state. If any nutrient is excessive, increasing the dietary fat content could improve performance. Knapka *et al.* (1977) found that increasing the fat content of a diet from 4 percent to 12 percent of the diet improved reproduction of mice when the diet contained 24 percent protein but decreased reproduction when the diet contained 18 percent protein. Thus, the effect on reproduction of adding fat to the diet appeared to be mediated through its effect on the concentration of protein per kilocalorie of metabolizable energy.

Essential Fatty Acids

Mice specifically require a dietary source of linoleic and/or arachidonic acids. The exact requirement for mice has not been determined. Menton (1970) used a concentration of 0.625 percent linoleic acid in an adequate control diet. In the absence of specific studies in the mouse, the concentration of 0.3 percent linoleic acid, as recommended for the rat, has been adopted.

A requirement for linolenic acid by the mouse was reported by Rivers and Davidson (1974) because mice fed safflower oil (low in linolenate) had a 17 percent lower weaning weight in the second generation than those fed soy or linseed oils. The second-generation mice also showed an increased fasting metabolic rate, which the authors considered probably indicative of essential fatty acid deficiency. However, Pleasants *et al.* (1970) maintained germfree mice through five generations on a diet containing only highly purified linoleate. A possible requirement for linolenate needs to be studied further with purified fatty acid derivatives. Safflower oil might have metabolic effects due to its high ratio of polyunsaturated to saturated fats.

Since natural fats and oils vary widely in their content of essential fatty acids, they cannot be substituted for one another in diets without determining the essential fatty acid content.

Signs of Deficiency Decker *et al.* (1950) described chronic essential fatty acid deficiency in mice as involving hair loss, dermatitis with scaling and crusting of skin, and occasional diarrhea. Deficiency in older mice caused infertility without visible skin changes. Menton (1968, 1970) observed scaling and inelasticity of the skin and extensive hair loss. The epidermis became thickened, with increased mitotic and histochemical activity. Despite its increased thickness, the deficient skin permitted greater transepidermal water loss, which appeared related to the wide separation of the epidermal filaments in the stratum corneum demonstrated by electron microscopy.

MINERALS

Since publication of the second revised edition of *Nutrient Requirements of Laboratory Animals* (NRC, 1972), considerably more information has become available on mineral nutrition of mice, although some very large gaps remain in our knowledge of requirements.

Calcium and Phosphorus

Reported calcium concentrations in mouse diets varied from 0.4 percent in a purified diet (Mirone and Cerecedo, 1947; Morris and Lippincott, 1941) to 2.1 percent in a natural-ingredient diet (NRC, 1972). A dietary calcium content of 1.2 percent in a natural-ingredient diet supported good reproduction in BALB/cAnN, C57BL/6N, N:NIH(S), C3H/HeN, and DBA/2N mice (Knapka *et al.*, 1974, 1977). A purified diet that contained 0.8 percent calcium supported normal growth and development (Bell and Hurley, 1973). Wolinsky and Guggenheim (1974) used 0.5 percent calcium in their adequate control diet for deficiency studies.

The phosphorus content of various mouse diets was reported to range from 0.3 percent to 1.2 percent (Mirone and Cerecedo, 1947; Morris and Lippincott, 1941). Good reproduction was obtained with five strains of mice fed a diet that contained 0.86 percent phosphorus (Knapka *et al.*, 1974, 1977). A purified diet promoting normal growth and reproduction in a hybrid line contained 0.4 percent phosphorus (Bell and Hurley, 1973).

The ratio of calcium to phosphorus in the diet is important for normal mineral metabolism, but the optimal ratio may change with age. Krishnarao and Draper (1972) reported that 1.2 percent dietary calcium induced bone resorption in aging mice when the dietary phosphorus level was 1.2 percent but not when phosphorus was 0.6 percent. Calcium to phosphorus ratios of 1.2:2.0 supported good reproduction, but a ratio of 2 seems advisable for the latter half of the life span.

Signs of Deficiency Wolinsky and Guggenheim (1974) and Ornoy *et al.* (1974) reported that a diet containing only 0.02 percent calcium decreased weight gain, bone ash, and serum calcium in Swiss mice. However, these effects were much less marked in mice than in rats, because the mice showed more effective compensatory mechanisms. Mice increased the concentration of calcium-binding protein in the duodenal mucosa to provide improved calcium utilization. They also compensated by reduced skeletal growth, so that growth reduction rather than osteoporosis was the more prominent sign of deficiency.

Chlorine (See Sodium)

Chromium

The chromium requirement of mice has not been established, but Schroeder *et al.* (1963) found that trivalent chromium significantly increased the growth and decreased the mortality of male mice in a metal-free environment when provided in the drinking water at a concentration of 5 mg/l. Since mice usually consume twice as much water as dry diet, their intake would be equivalent to that on a diet containing 10 mg/kg.

The metabolic role of chromium has been elaborated with studies in the rat. Based on the chromium content of published diets, the requirement for the mouse has been set at 2 mg/kg of diet for mice reared in ordinary (not metal-free) laboratory environments.

Copper

No studies of the copper requirement of the mouse have been reported. A natural ingredient diet that supported good growth and reproduction contained 16.1 mg of copper per kilogram of diet (Knapka *et al.*, 1974), while an adequate purified diet contained 4.5 mg/kg as added copper (Hurley and Bell, 1974).

Fluorine

The existence of a mouse requirement for fluorine is still controversial. Schroeder *et al.* (1968) reported that female mice given 10 mg/l of fluorine in their drinking water grew larger than those given none. Weber and Reid (1974), however, found no fluorine requirement for growth or reproduction in mice through six generations.

A fluoride requirement for mouse reproduction was reported by Messer *et al.* (1973); there was reduced fertility and maternal and neonatal anemia when the diet contained only 0.1 to 0.3 mg/kg fluoride, but reproduction was normal when 50 mg/l of fluorine was added to the drinking water. However, Tao and Suttie (1976), using a diet containing more copper and iron than that of Messer *et al.* (1973), found no requirement for fluorine. They suggested that the additional fluoride in the latter study had enhanced utilization of the marginally deficient copper and iron.

Iodine

No studies of the iodine requirements of mice have been reported. In an investigation of iodine deficiency in mice, Abbassi and McKenzie (1970) used a diet containing approximately 0.03 mg/kg to produce the deficiency state. Their control diets contained 0.19 mg/kg in early experiments and 0.25 mg/kg in later ones. A natural-ingredient diet known to provide good reproduction contained 1.9 mg/kg, while a purified diet supporting good growth and reproduction in mice contained 36 mg/kg (Table 10).

Signs of Deficiency Abbassi and McKenzie (1970) reported that an iodine-deficient diet fed to mice for six months resulted in thyroid glands 3 times normal size, pituitaries 2 times normal size, and serum thyrotropin concentrations 200 times normal. Serum concentrations and ratios of thyroid-related compounds showed decreases, as also observed in rats fed iodine-deficient diets.

Iron

Inoue (1932) found iron to be required for both growth and reproduction of mice, but only recently have quantitative data on requirements become available. Sorbie and Valberg (1974) compared growth and iron storage in male C57BL/6J mice fed various concentrations of iron in a purified diet.

Iron concentrations of 25 to 100 mg/kg of diet supported normal growth and hematopoiesis, but provided less storage iron than a natural-ingredient diet containing 220–240 mg/kg. A purified diet containing 120 mg/kg produced growth, hematopoiesis, and iron storage equivalent to the natural-ingredient diet, as did purified diets containing 240 and 480 mg/kg. Only the 120 mg/kg diet was tested for reproduction; it supported good reproduction through three generations. Two natural-ingredient diets known to provide good growth and reproduction in three mouse strains contained 198 and 255 mg/kg (Knapka *et al*., 1974). A purified diet that supported normal growth and development contained 299 mg/kg (Bell and Hurley, 1973).

Signs of Deficiency Inoue (1932) reported characteristic anemia signs, as well as reduced birth weights and litter sizes in iron-deficient mice.

Magnesium

Magnesium has been shown to be a dietary essential for the mouse, but the optimal level of its intake for this species has not been established. Alcock and Shils (1974) demonstrated deficiency signs in mice fed 0.002 percent dietary magnesium but not in those fed 0.04 percent. A purified diet containing 0.073 percent supported normal growth and development (Bell and Hurley, 1973). Natural-ingredient diets known to provide for good growth and reproduction in three mouse strains contained 0.180 and 0.260 percent (Knapka *et al*., 1974). Dubos *et al*. (1968) reported sudden death in some lactating female mice fed 0.05 percent, but not in those fed 0.07 percent. This parallels the finding in rats (Hurley *et al*., 1976) that the magnesium concentration in the diet must be higher for lactation than for growth.

Signs of Deficiency Alcock and Shils (1974) reported that magnesium-deficient mice, without showing previous hyperirritability, developed rapid and usually immediately fatal convulsions. Hypomagnesemia was positively correlated with hypocalcemia in mice fed their diet, which was relatively low in calcium. Soft tissue calcification resulting from magnesium deficiency has been reported in only one strain of mouse, the hereditarily diabetic KK strain (Hamuro *et al*., 1970).

Manganese

The minimum requirement for manganese in the diet of mice has not been established, but diets containing 45 mg/kg of this element were adequate for several genetic strains (Bell and Hurley, 1973; Hurley and Bell, 1974). Reproduction, growth of offspring, and survival to 30 days of age, as well as inner ear development, were as good in hybrid mice receiving this dietary level, as in mice fed a natural-ingredient diet containing approximately 50 mg/kg (Bell and Hurley, 1973). The 45 mg/kg concentration also produced normal morphogenesis of inner ear otoliths in three strains of inbred mice (Hurley and Bell, 1974).

Signs of Deficiency Dietary deficiency of manganese during growth and reproduction in female mice resulted, as in other species tested, in congenital ataxia in the offspring caused by abnormal development of the inner ear, especially failure of otolith formation (Erway *et al*., 1970). In addition, postnatal survival of the offspring was reduced, but litter size (Erway *et al*., 1970), birth weight (Hurley and Bell, 1974), and body weight gain of the young (Bell and Hurley, 1973) were not affected. Offspring fed the manganese-deficient diet into later life showed obesity and fatty livers, as well as abnormalities in ultrastructural parameters, including altered integrity of cell membranes, swollen and irregular endoplasmic reticulum, and abnormal mitochondria (Bell and Hurley, 1973). Genetic factors were found to influence the magnitude of the response of mice to dietary deficiency of manganese (Hurley and Bell, 1974).

Phosphorus (See Calcium)

Potassium

The potassium requirement of the growing mouse (Carworth Farms No. 1) has been found to be 0.2 percent of the diet (Bell and Erfle, 1958). A natural-ingredient diet known to support good growth and reproduction contained 0.82 percent potassium (Knapka *et al*., 1974). A purified diet promoting good growth and reproduction contained 0.89 percent potassium (Bell and Hurley, 1973).

Signs of Deficiency Mice fed highly purified diets, deficient only in potassium, died within one week, after having exhibited outward signs of inanition. Lusterless eyes and hair coat, dry scaly tail, and general emaciation were observed in connection with severe deficiency. Partial deficiencies resulted in poor growth and lack of "bloom" (Bell and Erfle, 1958).

Selenium

Spallholz *et al*. (1973) studied the effect of selenium supplementation on immunologic responses of Swiss-Webster weanling mice. The experiments suggest that primary immune responses were highest when the diet contained selenium at a level of 1.25 mg/kg and declined at higher or lower concentrations. They found that mice could survive concentrations of selenium as high as 40 mg/kg of diet it they were adapted to this content gradually. The metabolic roles of selenium and vitamin E have been shown to be interrelated in all species studied (Hoekstra, 1975), but the relationship in the mouse has not been investigated.

Sodium and Chlorine

Salt (NaCl) requirements do not appear to have been studied. The concentrations used in various diet formulas range from 0.5 to 1.0 percent of the diet. Two natural-ingredient diets known to provide for good growth and reproduction contained 0.36 and 0.49 percent sodium

(Knapka *et al.*, 1974). The purified diet promoting good growth and development contained 0.39 percent sodium (Bell and Hurley, 1973).

Zinc

Zinc is essential for the mouse (Bertrand and Bhattacherjee, 1934; Day and Skidmore, 1947; Nishimura, 1953). Two natural-ingredient diets promoting good growth and reproduction contained 50 and 58 mg/kg (Knapka *et al.*, 1974). A purified diet promoting good growth and reproduction was analyzed to contain 31 mg/kg (Bell and Hurley, 1973).

Signs of Deficiency Day and Skidmore (1947) found that an intake of only 3 mg/kg of diet resulted in deficiency signs, including loss of hair on shoulders and neck, emaciation, and decreased liver and kidney catalase activity.

Other Minerals

Huff *et al.* (1956) found that 3.75 mg/kg of bromine in the diet reversed the growth depression caused by feeding iodinated casein; they suggested that the low effective concentration of bromine indicated a nutritional rather than pharmacological role. Jaffe (1952) found a need for 0.2 percent dietary cobalt chloride ($CoCl_2$) to maintain reproduction through successive generations in mice fed only plant-derived materials. Since injected $CoCl_2$ was ineffective, they postulated that the cobalt was needed to support intestinal microbial biosynthesis of vitamin B_{12}. It is probably premature to conclude that these elements are required in ordinary laboratory situations. The same is also true of other elements (nickel, silicon, tin, and vanadium) found to be required by rats in ultraclean environments (see the chapter on rats).

FAT-SOLUBLE VITAMINS

Since publication of the last edition of *Nutrient Requirements of Laboratory Animals* (NRC, 1972), no new reports of studies designed to establish the requirements of fat-soluble vitamins for mice have been found.

Vitamin A

Vitamin A was shown by Wolfe and Salter (1931) to be required by the mouse. Morris (1947) estimated the daily requirement to be about 5 μg of β-carotene, or 0.3–0.6 μg of vitamin A. The vitamin A requirements for pregnancy and lactation are reported to be similar to those for growth (McCarthy and Cerecedo, 1952; Morris, 1947). Slanetz (1943) pointed out that vitamin A requirements for all species range from 25 to 39 mg/kg of body weight per day, a maximum of 200 μg/kg of diet. On the basis of these data, the stated requirement for vitamin A is 1–2 IU/day, or 250–500 IU/kg of feed.

Most mouse diets have contained considerably higher concentrations of vitamin A than those mentioned above. Slanetz (1943) found that various mouse diets ranged from 4,600 to 5,100 IU/kg of feed. Other formulations have been known to contain up to 60,800 IU/kg. The natural-ingredient diets used by Knapka *et al.* (1974, 1977), which produced acceptable reproduction in outbred and inbred mice, had vitamin A concentrations of 15,000 IU/kg of diet as compared to 4,000 IU/kg in the American Institute of Nutrition purified diet (American Institute of Nutrition, 1977), which also was adequate for mouse reproduction.

The susceptibility of vitamin A to oxidation must be considered when formulating diets. Zimmerman and Wostmann (1963) reported vitamin A activity was decreased by 20 percent as a result of steam sterilization.

Signs of Deficiency or Excess Vitamin A deficiency signs include tremors, diarrhea, rough hair coat, keratitis, abscesses, poor growth, rectal and vaginal hemorrhages, abortion, resorption, and permanent sterility in males. Vitamin A toxicity should also be kept in mind. Vitamin A administered in doses as small as 250 IU/day during critical phases of gestation resulted in toxicity as shown by serious reproductive disturbances and malformation of embryos (Giroud and Martinet, 1959, 1962).

Vitamin D

Beard and Pomerene (1929) found that mice were susceptible to rickets. Bell (1972) fed a natural-ingredient diet that contained 150 IU/kg of vitamin D to many generations of albino mice (CF No. 1) with no evidence of deficiency. The vitamin D concentration in natural-ingredient diets, which support acceptable reproduction in mice, is approximately 5,000 IU/kg of diet. This concentration appears to be the same for all the most readily available diets. The purified diet recommended by the American Institute of Nutrition (1977) contains 1,000 IU/kg.

Vitamin E

Bryan and Mason (1940) observed fetal resorption in vitamin E-deficient female mice similar to that observed in rats, but found no evidence of testicular injury in the vitamin E-deficient males. They reported that 350 μg of α-tocopherol daily was the minimum required for a normal first pregnancy. This corresponds to a dietary concentration of about 70 mg/kg. In contrast, Goettsch (1942) showed that 0.5–1.0 mg of α-tocopherol given at the onset of gestation would suffice. The natural-ingredient diets used by Knapka *et al.* (1974, 1977) contained 36.7 mg of α-tocopherol per kilogram. Some commercially available mouse diets contain over 65 mg/kg.

Signs of Deficiency In life-span studies, Lee *et al.* (1962) found that vitamin E deficiency resulted in convulsions and heart failure, but that vitamin B_{12} and mineral supplementation were modifying factors. Pappenheimer (1942) reported muscular dystrophy and hyaline degeneration in vitamin E-deficient mice, but at a lower incidence than was observed in rats. Cerecedo and Vinson (1944) concluded that vitamin E was related to protein metabolism; they found that muscular paralysis could be prevented either by increasing the protein level in the diet or by including 20 mg of α-tocopherol per kilogram of diet. Work with other species indicates that selenium or sulfur-containing amino acids may have been involved in the effect of the increased protein content (Hoekstra, 1975), as selenium can replace vitamin E in some of its functions. Bruce (1950) found α-tocopherol to be effective in reducing the mortality in litters whose dams were fed diets containing 2 percent cod-liver oil.

Vitamin K

In general, vitamin K is not added to diets for conventionally reared mice. Adequate amounts of vitamin K to meet body requirements are synthesized by intestinal microorganisms. Woolley (1945) reported that vitamin K corrected vaginal hemorrhages and fetal resorptions induced by administration of DL-α-tocopherol quinone, an analog of vitamin K.

Mice maintained in germfree or SPF environments or treated with antibacterial drugs require 3 μg of supplemental vitamin K per day, which corresponds to a dietary concentration of 0.7 mg/kg (Wostmann, 1975). Vitamin K deficiency has been implicated in outbreaks of hemothorax affecting male mice of various strains maintained in SPF environments at the National Institutes of Health.

WATER-SOLUBLE VITAMINS

Ascorbic Acid

The successful maintenance of many mouse colonies on diets devoid of ascorbic acid has fully confirmed the demonstration by Ball and Barnes (1941) that the mouse requires no dietary source of vitamin C.

The B Vitamins

Since the last edition of this volume (NRC, 1972), there have been no new quantitative determinations of minimal requirements for B vitamins by mice. The previous requirements are again listed in Table 10. Table 11 lists the B vitamin content of three purified diets, a natural-ingredient diet, and a chemically defined diet fed to germfree mice. The last may be considered an upper limit for requirements, as there is no microbial synthesis of vitamins.

The results of Luckey *et al.* (1974) indicated that the B vitamin requirements listed in the previous volume (NRC, 1972) may be greater than necessary for conventionally reared mice. Mice were fed an irradiated Apollo space diet containing 20 to 40 percent of the recommended concentrations of folacin, pantothenic acid, riboflavin, and thiamin. These levels were adequate for growth and reproduction of conventional CRL-CD1 mice, but did not support reproduction under germfree conditions. Lemonnier *et al.* (1974) fed Swiss (cesal) mice approximately one-fourth the recommended concentrations (NRC, 1972) of niacin, pantothenic acid, riboflavin, thiamin, and vitamin B_6 in a diet that contained 42 percent saturated fat and was designed to encourage obesity in this normally nonobese strain. The mice grew normally and were not obese, but became so when the B vitamin concentrations were increased. Knapka (unpublished data) found that autoclaved diets containing as little as 0.6 mg/kg thiamin after autoclaving (8 mg/kg before) supported normal reproduction for two generations in inbred and outbred strains of SPF barrier-sustained mice.

Biotin

Nielsen and Black (1944) showed that mice required a supplement of biotin when fed a chemically defined basal diet that had supported growth of rats. Dietary sulfasuxidine accentuated the deficiency. Mirone and Cerecedo (1947) found that biotin added to a purified diet improved reproduction and lactation. Fenton and Cowgill (1948) and Fenton *et al.* (1950) used 20 μg biotin/kg of diet. Adequate diets (Table 10) contain 0.2 to 1.0 mg/kg of diet, but the actual requirement has not been determined.

Signs of Deficiency These include alopecia, achromotrichia, and growth failure, as well as decreased reproduction and lactation efficiency (Nielsen and Black, 1944).

Choline

Choline was first recognized as a dietary essential for the mouse by Best *et al.* (1934), who observed fatty livers in choline-deficient mice. Adequate diets (Table 11) contain 750 to 4,000 mg/kg of diet. Establishing a minimal choline requirement is not now possible, because in earlier studies wide variations in concentration were fed and because choline can be synthesized from methionine (see the chapter on the rat).

Signs of Deficiency Choline-deficient mice have shown fatty livers with nodular parenchymal hyperplasia, myocardial lesions, lowered conception rates in females, and low viability of the young (Buckley and Hartroft, 1955; Meader and Williams, 1957; Mirone, 1954; Saucier and Demers, 1958; Williams, 1960). In contrast to earlier descriptions of fibrosis, Rogers and MacDonald (1965) observed that deficient C57BL mice, unlike rats, did not develop cirrhosis or fibrosis of the liver, but only fatty livers. There were acute and chronic inflammation and necrosis of individual hepatic cells. Proliferation of parenchymal cells increased with fat deposition. There was increased thymidine uptake by endothelial, perivascular, and parenchymal cells. Fifty-four percent of the choline-deficient mice died during a 24-week period.

Folic Acid

Nielsen and Black (1944) and Weir *et al.* (1948) demonstrated the essential nature of folic acid for the growing mouse. Cerecedo and Mirone (1947) reported that folic acid increased survival to weaning of suckling mice from 34 percent (basal diet) to 69 percent. Mirone and Cerecedo (1947) added 5 mg of xanthropterin to a 100-g basal diet and increased the percent of young weaned from 41 to 83 percent. Fenton *et al.* (1950) obtained satisfactory growth in mice fed purified diets containing 0.5 mg/kg of folic acid. Table 11 shows that folic acid concentrations in the range 0.5 to 4.0 mg/kg were adequate. Minimum requirements have not been determined, but the lowest concentration in adequate diets (0.5 mg/kg) has been recommended in Table 10.

Signs of Deficiency or Excess Weir *et al.* (1948) observed the following effects of 50 days of a folate-deficient diet: decrease in white cell count from a normal 6,000 to less than 4,000/mm^3; decrease in red cell count from 10,000,000 to as low as 4,000,000/mm^3; disappearance of megakaryocytes and nucleated cells from the spleen and accumulation of hemosiderin; and packing of the bone marrow with large immature cells and virtual disappearance of normal cell types. They concluded that the pattern was one of maturation arrest. Rothenberg *et al.* (1973) observed an impaired antibody response in folate-deficient mice that persisted after folate repletion. Shaw *et al.* (1973) observed decreased growth, especially of brain and liver, in young mice deprived of folate both pre- and postnatally. Addition of sulfamethazine to the diet accentuated the deficiency and caused death within 5 days.

Auletta *et al.* (1974) injected subcutaneously into mice 300 mg of folate per kilogram of body weight and observed precipitation within the kidney followed by hyperplasia, doubling of kidney weight, and some deaths within the first 4 days. Their studies indicated this was a nonspecific effect of folate due to mechanical injury by the precipitate.

Inositol (Myo-inositol)

Woolley (1941) described a dietary deficiency characterized by cessation of growth and loss of hair; he reported that inositol was the antialopecia factor, because 100 mg/kg of diet cured the condition. Later (1942) he demonstrated intestinal microbial synthesis of inositol, which could account for individual variability in response. Other studies (Cerecedo and Vinson, 1944; Fenton *et al.*, 1950; Martin, 1941; Shepherd and Taylor, 1974a) did not confirm the essentiality of inositol for growth of mice. Recent studies in some other rodent species indicate that they require inositol under conditions of microbial suppression and special physiological stress. Shepherd and Taylor (1974b) found that inositol enhanced intestinal lipid transport in rats fed a 31 percent fat diet. Burton and Wells (1977) observed that rats fed 0.5 percent phthalysulfathiazole in the diet required inositol to prevent fatty liver during lactation; 500 mg/kg of diet was sufficient. Hegsted *et al.* (1974) reported an inositol requirement by female gerbils that was increased by feeding saturated fat (coconut oil) or cholesterol. Anderson and Holub (1976) found that either tallow or the highly unsaturated rapeseed oil caused liver fat accumulation in inositol-deficient rats fed succinyl sulfathiazole, whereas corn oil or soybean oil did not; 0.5 percent inositol was protective.

If SPF, gnotobiotic, germfree, or antibiotic-treated mice are fed diets with the above types of fat, then inositol may be required in the diets. A purified diet fed germfree rats and mice contained 1,000 mg/kg (Wostmann and Kellogg, 1967). Chemically defined diets that supported growth and limited reproduction in germfree CFW or C3H mice contained 238 mg/kg (Pleasants *et al.*, 1970, 1973). A concentration of 500 mg/kg of diet was adequate for any combination of antibiotics, lactation, and unusual fat intake in rats and gerbils and appeared to be the upper limit of the inositol requirement. However, conventionally reared mice fed ordinary diets have not been found to require dietary inositol since the early studies of Woolley (1941, 1942).

Niacin

No reports on a niacin requirement are available. Calculations and reported analyses of natural-ingredient diets revealed niacin concentrations of 48 to 143 mg/kg of diet. Niacin concentrations of 50–55 mg/kg were used successfully in purified diets (Bell, 1972), but these concentrations probably exceed the minimum requirement. Satisfactory diets have contained from 5 mg/kg (John and Bell, 1976) to 80 mg/kg (Knapka *et al.*, 1974). The concentration of 10 mg/kg set in the previous edition of this volume (NRC, 1972) appears satisfactory.

Pantothenic Acid

The pantothenic acid requirement for growth of two strains of mice was 30 μg per day (Morris and Lippincott, 1941; Sandza and Cerecedo, 1941). This requirement, corresponding to 7.5 mg/kg of diet, was confirmed by Fenton and Cowgill (1947a, 1948) and Fenton *et al.* (1950) with one strain of mice, but maximum growth was not obtained with all strains at this concentration.

The reproduction and lactation requirements for this vitamin have not been reported, but various natural-ingredient diets contain from 10 to 26 mg/kg, and the satisfactory diets of Table 10 contain 16 to 50 mg/kg. The concentration of 10 mg/kg suggested in the previous edition (NRC, 1972) probably is adequate, but there may be strain differences in the requirement.

Signs of Deficiency The following pantothenic-acid-deficiency signs in growing mice were reported by Morris and Lippincott (1941): loss of weight; loss of hair, particularly on the ventral surface, flanks, and legs; dermatosis; partial posterior paralysis; other neurological abnormalities; and achromotrichia.

Riboflavin

Riboflavin requirements for normal growth appear to be about 4 mg/kg of diet (Fenton and Cowgill, 1947a,b; Wynder and Kline, 1965). Most satisfactory diets (Table 10) provide 7 mg of riboflavin per kilogram of feed, and this concentration is therefore suggested as adequate for reproduction and lactation.

Signs of Deficiency Ariboflavinosis in the mouse was described by Lippincott and Morris (1942). They reported the development of either atrophic or hyperkeratotic epidermis with normal sebaceous glands, myelin degeneration in the spinal cord, and corneal vascularization with ulceration. Morris and Robertson (1943) found that adult mice lost weight and young mice grew poorly on diets containing 0.4–0.6 mg/kg of riboflavin and died within 9 weeks. Kligler *et al.* (1944) showed that riboflavin-deficient mice had lowered resistance to *Salmonella* infection. Hoppel and Tandler (1975) reported striking increases in the size of hepatic mitochondria and a greatly decreased capacity for ADP-stimulated respiration in riboflavin deficiency. In some animals, the livers were yellow and the cytoplasm of the cells was engorged with small lipid droplets. In other animals, the livers were redder than normal, and their hepatocytes contained few lipid droplets.

Thiamin

Hauschildt (1942) established the minimum requirement of thiamin for normal growth of mice at 10 μg per day. This would correspond to a concentration of about 3 mg of thiamin per kilogram of diet. Morris and Dubnik (1947) later found the growth requirement to be about 4–6 μg of the vitamin per day in mice fed a diet containing 22 percent fat. No studies on the specific requirements for reproduction and lactation have been reported, but Mirone and Cerecedo (1947) found that 20 mg/kg of diet were adequate.

The requirements for these functions are probably close to those for growth, since several satisfactory diets contain less than 20 mg/kg. Natural-ingredient diets reported by Bell (1972), the *Handbook of Laboratory Animals* (NRC, 1954), and Rockland Farms (Anonymous, 1958) contain, by calculation from published tables of vitamins in feedstuffs (NRC, 1964), about 5.5, 4.6, and 2.2–4.6 mg of thiamin, respectively, per kilogram of diet. The satisfactory diets in Table 10 contained 5–23 mg/kg. The concentration of 5 mg/kg set in the previous edition (NRC, 1972) is therefore retained.

Signs of Deficiency Morris (1947) and Jones *et al.* (1945) reported violent convulsions, especially when the animal was held a few seconds by the tail; cartwheel or circular movements; brain hemorrhages; decreased food intake; poor growth; early mortality; silvery-streaked muscle lesions; and testicular degeneration. The onset of ataxia in thiamin-deficient Swiss-Webster mice was preceded by a rapid rise in brain α-ketoglutarate (Seltzer and McDougal, 1974).

Vitamin B_6 (Pyridoxine, Pyridoxal, Pyridoxamine)

According to Miller and Baumann (1945) and Morris (1947), mice grew satisfactorily when fed diets containing 1 mg of pyridoxine per kilogram of diet. Pyridoxamine and pyridoxal were found to be less active than pyridoxine. Bell *et al.* (1971) found 0.2 mg/kg of pyridoxine to be growth-limiting for two strains, whereas 8.2 mg/kg supported normal growth. Satisfactory diets (Table 10) contain from 4.0 to 22.5 mg/kg. The concentration of 1 mg/kg set by Miller and Baumann (1945) and by Morris (1947) appears to be adequate.

Signs of Deficiency Deficiency signs include poor growth, hyperirritability, posterior paralysis, necrotic degeneration of the tail, and alopecia (Beck *et al.*, 1950). Recent investigators (Keyhani *et al.*, 1974) observed in B_6-deficient CF1 mice a progressive hypochromic microcytic anemia with hypersideremia. It was accompanied by an increase in reticulocyte count not observed in vitamin B_6 deficiencies of other species.

Vitamin B_{12}

Jaffé (1952) reported that vitamin B_{12} was required in excess of 5 μg/kg of diet for growth and between 4 and 5 μg/kg for reproduction and lactation. Lee *et al.* (1962) demonstrated its necessity for gestation. Bosshardt *et al.* (1950) found that it improved growth and survival of mice fed low-fat, high-protein diets, or diets containing 0.5 percent iodinated casein. Meites (1952), however, observed that vitamin B_{12} decreased the stimulating effect on growth produced by a diet containing 0.025 percent iodinated casein.

Satisfactory diets (Table 11) provided 0.01 to 0.58 mg of vitamin B_{12} per kilogram of diet. Because of the contribution of vitamin B_{12} made by intestinal bacteria, no definite requirement can be set. The lowest concentration in the above satisfactory diets has been set as a requirement. This is done primarily to call attention to the possibility that vitamin B_{12} might be required under conditions such as antibiotic feeding, germfree environment, or coprophagy prevention, which could reduce the availability of bacterially synthesized vitamin B_{12}.

Signs of Deficiency Young mice deficient in vitamin B_{12} show retarded growth and renal atrophy (Lee *et al.*, 1962). Deficiency causes death of young both before and after birth.

WATER

Mice should be provided with a readily available supply of water. Very little specific information on requirements seems to have been published (Green, 1966); and, while statements have appeared to the effect that no drinking water is needed, it is probable that the use of high-moisture foods, such as vegetables and wet mashes, led to such deductions. Restriction of water intake results in decreased voluntary food consumption (Chew and Hinegardner, 1957). Dalton (1963) reported that addition of 30 percent cellulose to a diet can increase water intake up to 50 percent. Environmental temperature is undoubtedly a factor affecting water requirements. Mice fed dry rations and housed at temperatures of 75°–80°F may die if deprived of water for a day.

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NUTRIENT REQUIREMENTS OF THE GERBIL

Use of gerbils has increased during the past decade because of their various unique characteristics. The gerbil is one of many rodents in the subfamily Gerbillinae and might more properly be termed "clawed jird." Those in the United States originated in or near Mongolia, were introduced from Japan, and are of the species *Meriones unguiculatus*. Those in Europe originated in Tunisia and are *M. libicus* or *M. shawi*. Other species and subspecies exist. Many investigators claim the gerbil to be superior to the rat as a model in experiments related to human health. Some subject areas related to medical sciences wherein gerbils have been used successfully are listed below, together with selected references:

1. Behavior (Brosgole *et al.*, 1976; Cullen and Harriman, 1973; Harriman, 1969b, 1974; Rich, 1968; Robbins, 1976; Schwentker, 1971; Thiessen *et al.*, 1968; Walters *et al.*, 1963).
2. Lipid, lipoprotein, and cholesterol metabolism (Bazzano and Bazzano, 1972; D'Elia *et al.*, 1972; Gordon and Cekleniak, 1961; Gordon and Mead, 1964; Hegsted and Gallagher, 1967; Hegsted *et al.*, 1973; Kroes *et al.*, 1973; Nicolosi *et al.*, 1976; Rich, 1968; Roscoe and Fahrenbach, 1962; Seiler *et al.*, 1971a,b; Wong *et al.*, 1965).
3. Atherogenesis (Gordon and Cekleniak, 1961; Gordon and Mead, 1964; Oliver and Peron, 1964; Wexler, 1974; Wexler *et al.*, 1971).
4. Epilepsies (Harriman, 1974; Loskota *et al.*, 1974; McCarty, 1975; Robbins, 1976; Thiessen *et al.*, 1968).
5. Hormone regulation and organ response (Burns, 1956; Boquist, 1972; Glenn and Gray, 1965; Hummell, 1963; Oliver and Peron, 1964).
6. Thermal regulation (McManus, 1971; Mele, 1972; Robinson, 1959).
7. Responses to pathogenic agents (Schwentker, 1971; Thompson, 1976; Tripathy and Hanson, 1976).
8. Diet and nutrition (Arrington, 1968; Arrington *et al.*, 1973; Boquist, 1972; Cullen and Harriman, 1973; Hall and Zeman, 1968; Harriman, 1969a, 1974; Hegsted *et al.*, 1973, 1974; Kroes *et al.*, 1973; McManus, 1972; Mele, 1972; Rich, 1968; Schwentker, 1971; Winkelmann and Getz, 1962; Zeman, 1967).
9. Growth reproduction and development (Loew, 1968; Marston and Chang, 1965; McManus and Zurich, 1972; Norris and Adams, 1972; Rich, 1968; Schwentker, 1971).
10. Resistance to radiation, xenobiotics, hypoxia, and ischemia (Chang *et al.*, 1964; Dixit *et al.*, 1976; Ito *et al.*, 1976; Karel and Saxena, 1976; Kroot *et al.*, 1976).
11. Genetics (Loskota *et al.*, 1974; McCarty, 1975; Robbins, 1976; Thiessen *et al.*, 1968).
12. Intermediary metabolism (Boquist, 1972; Nicolosi *et al.*, 1976; Seiler *et al.*, 1971a,b).

Excellent articles on general feeding and care of this species have been published, but only a few deal with specific nutrient requirements (Marston and Chang, 1965; McManus, 1971; Rich, 1968; Schwentker, 1971).

GENERAL DEVELOPMENT

Gerbils have been bred in captivity since before 1935. Adults weigh 70 to 135 g and have 4 to 5 pups per litter with birth weights of about 3 g. They are usually weaned at 21–24 days of age weighing 14–18 g. An acceptable weight gain is 1 g/day from 3.5 to 7.0 weeks of age. Sexual maturity is at 65 to 85 days. These animals exhibit polyestrus, postpartum mating with delayed implantation, and are usually monogamous. The gestation period is about 25 days. No light pattern need be maintained for normal reproduction, but one is usually followed (Arrington *et al.*, 1973; Loew, 1968; Marston and Chang, 1965; McManus, 1971; McManus and Zurich, 1972; Norris and Adams, 1972; Rich, 1968; Schwentker, 1971).

ENERGY AND WATER

Gerbils generally have had acceptable growth and reproduction when fed other rodent diets, such as rat, mouse, or guinea pig pelleted natural-ingredient diets. Several investigators used supplementary cereals and/or seeds (Arrington, 1968; Loew, 1968; Marston and Chang, 1965; McManus, 1971, 1972; McManus and Zurich, 1972; Mele, 1972; Norris and Adams, 1972; Rich, 1968; Schwentker, 1971; Loskota *et al.*, 1974). Growing gerbils consume about 5 to 6 g dry diet per day or 8 to 10 g of diet per 100 g of body weight. Dietary intake averaged 36 to 40 kcal of gross energy per 100 g of body weight per day (Harriman, 1969a; McManus and Zurich, 1972; Mele, 1972). Digestibility of energy was 93 to 94 percent when ambient temperature was 0° to 15°C, and both intake and digestibility decreased at temperatures of 20° to 35°C (Mele, 1972). Gerbils have been reported to live on cereal or pearled barley alone (Burns, 1956), but Rich (1968) observed high mortality when the animals were restricted for long periods to dry-type diets without water or green feeds such as carrots or lettuce.

Gerbils fed dry diets only excrete low volumes of highly concentrated urine—3.4 *M* urea per liter and 1.6 *N* NaCl (Burns, 1956). Green forage and free access to water should be provided with dry diets (Harriman, 1972; Marston and Chang, 1965; McManus, 1971; Mele, 1972; Norris and Adams, 1972; Rich, 1968; Seiler *et al.*, 1971a,b). Gerbils will voluntarily consume 4 to 10 ml water per 100 g of body weight daily (Harriman, 1969a; McManus, 1972; Winkelmann and Getz, 1962). McManus (1972) reported that total water (free + food + metabolic) averaged 13 percent of body weight.

FAT

Purified diets fed to gerbils contained 2 to 20 percent fat (Arrington, 1968; Arrington *et al.*, 1973; Harriman, 1969a; Hegsted *et al.*, 1973, 1974; Kroes *et al.*, 1973; Zeman, 1967). Growth response to variable amounts of dietary fat has not been quantitated, since most investigations emphasized and measured other criteria. Gerbils (50 g) gained more weight when fed a diet of 12 percent fat and 32 percent casein (percentages obtained from other gerbils offered a self-selection diet) than did similar gerbils fed a natural-ingredient diet (Harriman, 1969a). The minimum dietary fat requirement for maximum growth has not been determined.

Arachidonic acid is virtually absent from plasma cholesterol esters of gerbils and is comparatively low in body fat, yet the gerbil converts linoleic acid to arachidonic acid. Body fat of gerbils is higher in oleic and palmitic acid than is rat body fat (Gordon and Mead, 1964).

High dietary cholesterol leads to excess deposits in several body organs, but no overt plaque formation. However, older breeder animals on natural-ingredient diets show spontaneous arteriosclerosis (D'Elia *et al.*, 1972; Hegsted and Gallagher, 1967; Gordon and Cekleniak, 1961; Wexler *et al.*, 1971).

PROTEIN

Greater weight gains were obtained when weanling gerbils were fed more than 16.2 percent protein than when fed 12 to 14 percent (Table 12). Purified diets have contained casein or soy protein isolates. Body weight gains were 0.81 to 0.88 g/day for gerbils weighing 38 g initially when fed 20, 25, or 30 percent casein. Those fed 15 percent casein gained only 0.69 g/day ($P < 0.05$) (Hall and Zeman, 1968). The protein requirement of growing gerbils is at or above 16 percent, and protein calories as percent of total calories should be above 16 based on data of Arrington *et al.* (1973) and Hall and Zeman (1968). Diets near or below this requirement were used in some experiments (Hegsted *et al.*, 1973, 1974; Nicolosi *et al.*, 1976).

MINERALS

Many colonies of captive gerbils have been noted to have a low incidence (<2 percent) of convulsive seizures. Such seizures can be initiated by environmental changes, such as handling or other stimulation (Harriman, 1974; Loskota *et al.*, 1974; Marston and Chang, 1965; McCarty, 1975; Thiessen *et al.*, 1968; Zeman, 1967). The seizure incidence was altered by a combination of low dietary magnesium and handling or placing low-magnesium gerbils in a novel environment (Harriman, 1974). Gerbils fed a low-magnesium diet had a seizure incidence of 20 percent when placed in the novel environment between 12 and 32 days on

TABLE 12 Amounts of Dietary Protein and Fat in Purified Diets for Growing Gerbils

Initial Gerbil Weight (g)	Dietary Protein (%)	Dietary Protein (Cal% Pro)[a]	Gain (g/day)	Dietary Fat (%)	Investigator
18	16.2-20.8	16-21	1	5	Arrington *et al.*, 1973
18	12.3-14.2	12-14	0.6-0.8	5	Arrington *et al.*, 1973
18	18.0-24.0	15-25	1	4-12	Arrington, 1968
38	17-25	19-28	0.81-0.88	2	Hall and Zeman, 1968
38	13	14	0.69	2	Hall and Zeman, 1968
50	26	28	0.7	12	Harriman, 1969a
50	25-29	27-32	0.4	2	Zeman, 1967
50	12.8	17	0.4-0.48	20	Hegsted *et al.*, 1974

[a] Protein calories as percent of total dietary calories.

the diet. Gerbils fed the same diet but remaining in their cages had no seizures during this time. Magnesium was added to the diet at the rate of 1.39 g/kg, and gerbils fed this diet had no seizures when placed in a novel environment during the same period. Mortality was 25 percent in the magnesium-deprived, handled group, only 5 percent in the magnesium-deprived group not handled, and zero in the controls. The combination of low magnesium plus handling in a novel environment increased seizure activity. Gerbils fed diets low in calcium, sodium, or vitamin B_6 treated similarly had no seizures. These purified diets were purchased and presumably were formulated as described by Zeman (1967), omitting the nutrient in question (Harriman, 1974). Selective breeding has produced strains with different seizure incidence (Loskota *et al.*, 1974).

Gerbils fed a purified diet with 100 mg or less of magnesium per 100 g diet developed some degree of alopecia with severity related to the extent of magnesium deprivation. Alopecia became noticeable after 14 days when dietary magnesium was less than 12 mg/100 g. Mortality was 100 percent in 40 days when no magnesium was added to the diet and 70 to 83 percent when dietary magnesium was 6 to 12 mg/100 g. Dietary magnesium at a concentration of 25 mg/100 g prevented death and weight loss (Harriman, personal communication, 1976). The dietary requirement is suggested to be above 100 mg/100 g diet and perhaps as high as 200 mg/100 g.

A purified diet, without NaCl added, produced alopecia in 30 days without weight loss. Recovery was dramatic when NaCl was provided (Cullen and Harriman, 1973; Harriman, personal communication). Body weight of gerbils was stable when receiving 0.75 *M* salt solutions as the only liquid. Gerbils even obtained some metabolically useful body water when given only 1.25 *M* NaCl plus a dry diet, due to concentration of solute by kidneys. Feed intake declined progressively when the water contained 0.5 to 1.5 *M* NaCl (McManus, 1972).

Amounts of minerals supplied in natural-ingredient rodent diets commonly fed to gerbils apparently contain sufficient amounts of minerals. Amounts used in purified diets allowing satisfactory growth are shown in Table 13.

VITAMINS

Male gerbils fed diets containing about 2 percent fat do not have a dietary requirement for inositol, due to testicular synthesis of that vitamin. Female gerbils require more than 2 mg/100 g of diet. This requirement is increased to 7 mg/100 g when diets contain 20 percent unsaturated fat. The male requires inositol when fed this same amount of unsaturated fat, but the amount has not been determined. Addition of sufficient inositol to these diets prevented body weight loss or decreased gain, hyperkeratosis of the skin and accumulation of fat in the intestinal tissue, and increased inositol content of intestinal tissue (Hegsted *et al.*, 1973, 1974; Kroes *et al.*, 1973).

With dietary riboflavin concentrations of 0.046 to 0.070 mg/100 g of diet, growth rate and urinary riboflavin levels were less than with diets containing 0.35 to 0.77 mg/100 g. Urinary riboflavin over a 117-day period was greatest when gerbils were fed 0.63 mg/100 g, but was 32 and 5 percent of that value when they received 0.21 or 0.07 mg/100 g (Hall and Zeman, 1968). A requirement of 0.35 mg/100 g is suggested.

Inclusion of 0.2 to 0.5 percent cholesterol in a purified

TABLE 13 Amounts of Minerals and Vitamins in Purified Diets Fed to Gerbils by Investigators Where Acceptable Growth Rates Were Observed

Mineral Element	Amount per Kilogram of Diet	Vitamins	Amount per Kilogram of Diet
Calcium, mg	5,700–8,270	Thiamin, mg	4.0–22.5
Phosphorus, mg	3,270–4,000	Riboflavin, mg	3.7–20.0
Potassium, mg	7,200–8,770	Pyridoxine, mg	4.0–22.5
Magnesium, mg	930–1,670	Calcium pantothenate, mg	25–60
Sodium, mg	1,530–3,970		
Chlorine, mg	2,340–8,400	Choline, mg	750–3,000
Iron, mg	130–470	Niacin, mg	22.5–90.0
Copper, mg	0.40–4.5	Menadione, mg	0.1–45.0
Manganese, mg	3.30–45.0	*p*-Aminobenzoic acid, mg	0–100
Zinc, mg	0–8.4		
Iodine, mg	1.4–37.0	Inositol, mg	20–375
Fluorine, mg	0–11	Ascorbic acid, mg	0–900
		Folic acid, mg	100–1,800
		Biotin, μg	200–400
		Cobalamine, μg	0.18
		Vitamin A, IU	18,000–32,500
		Vitamin D, IU	2,000–3,250
		Vitamin E, IU	9–1,200

SOURCES: Arrington *et al.*, 1973; Hall and Zeman, 1968; Hegsted *et al.*, 1973, 1974; Kroes *et al.*, 1973; Zeman, 1967.

diet containing 20 percent fat increased intestinal fat. Additional dietary inositol prevented accumulation of gut fat, but allowed plasma cholesterol to increase. Cholesterol added to the diet apparently increased the need for additional dietary inositol (Hegsted *et al.*, 1974; Kroes *et al.*, 1973).

Natural-ingredient rodent diets fed to gerbils apparently furnish sufficient vitamins. Amounts used in purified diets allowing growth of 1 g/day for 18-g gerbils or 0.5 g/day for 50-g gerbils are shown in Table 13. The amounts listed for magnesium, riboflavin, and inositol are below or at the suggested requirements of 200, 0.35, and 2 mg/100 g of diet, respectively.

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NUTRIENT REQUIREMENTS OF THE GUINEA PIG

INTRODUCTION

The domestic guinea pig (*Cavia porcellus*) has been bred in captivity for at least 400 years and probably originated in Peru, Argentina, or Brazil (Weir, 1974). It has some anatomical peculiarities that should be mentioned before discussing nutrient requirements. Instead of canine teeth, it has an extensive diastema between the incisors and molars. The guinea pig, like other species of rodents, has open-rooted incisors that grow continuously throughout life. It is a monogastric herbivore, and the gastric mucosa does not have a keratinized portion. Another notable feature is the presence of a large semicircular cecum with numerous lateral pouches. This organ resembles that of the rabbit and possibly has similar digestive functions, e.g., synthesis of B vitamins by microorganisms and recycling of intestinal contents by coprophagy (Hunt and Harrington, 1974).

The guinea pig has a long gestation period (59–72 days), which accounts for its advanced development at birth. Newborn animals can consume semisolid and solid food immediately. In its native habitat, the guinea pig's diet consists of green vegetation and fruits; whereas in the laboratory the diets are generally of much higher caloric density and lower fiber content. Furthermore, the guinea pig eats many small meals throughout the day, may be selective in its choice of diets, and may resist abrupt changes in formula or form of diet. These behavioral characteristics and certain special nutritional requirements must be considered when designing nutritional or metabolic studies.

The guinea pig is best known from a nutritional standpoint because of its requirement for dietary vitamin C. This feature has made them particularly useful in studies of collagen synthesis, wound healing, and bone growth. The young guinea pig seems to have a relatively high requirement for arginine and folic acid. These characteristics and others mentioned above are discussed in greater detail in *The Biology of the Guinea Pig* (Wagner and Manning, 1976).

ENERGY

Experiments have not been done to determine actual energy requirements of this species. In a series of experiments designed to study feeding behavior, Hirsch (1973) found that the guinea pig is relatively insensitive to caloric density of the diet; up to 50 percent dilution of an adequate diet with finely ground cellulose did not lead to a compensatory increase in food intake. Instead, after some initial loss, body weight was maintained on less than 40 percent of the calories consumed prior to dilution by cellulose. These results indicate possible improved utilization of energy and cellulose. The cecum of the guinea pig contains short-chain fatty acids in concentrations comparable to those found in the rumen (Henning and Hird, 1970), and digestion of cellulose in this organ may contribute to meeting energy requirements.

Various carbohydrates have been used as energy sources and bulk in purified diets. Among these have been sucrose, glucose, cornstarch, gum arabic, wood pulp, agar, cellulose, and cellophane (Everson *et al.*, 1959; Navia and Lopez, 1973; Reid and Briggs, 1953; Roine *et al.*, 1949). Guinea pigs accepted the meal form of these diets poorly. Palatability was improved by pelleting. Navia and Lopez (1973) prepared their purified diet in gel form. By this procedure the diet was made palatable to young and adult guinea pigs, and nutritionally adequate, as shown by body weight gains. Digestible energy of these purified diets appears to be near 3.0 to 3.25 kcal/g of dry matter.

AMINO ACIDS AND PROTEINS

Woolley and Sprince (1945) observed that the guinea pig had an unusually high protein requirement when casein was used in a purified diet. The best weight gains occurred when the diet contained 30 percent casein. Arginine, cystine, and glycine added to an 18 percent casein diet produced equivalent growth. These results were confirmed and extended to demonstrate that the most limiting essential amino acid in casein for the guinea pig was arginine (Heinicke *et al.*, 1955, 1956; Reid and Mickelsen, 1963). As the casein content was decreased below 30 percent, arginine, methionine, and tryptophan became limiting in that order. Plant proteins contain generous amounts of arginine, and the guinea pig, a herbivore, grows well on diets that contain 18 percent protein from plant sources (Shelton, 1971). The young guinea pig not only has a high requirement for arginine, but arginine present in casein is probably only 70 percent available (Heinicke *et al.*, 1956). This estimate was verified in later studies, which showed that supplementation of a 30 percent casein diet (1.26 percent arginine) with 0.3 percent L-arginine resulted in improved growth, although 30 percent casein should theoretically meet the arginine requirement (Reid and Mickelsen, 1963).

Purified soybean proteins are used widely in experimental diets for guinea pigs. Such diets are adequate in arginine, but limiting in methionine at levels below 30 percent (Reid, 1966; Reid and Mickelsen, 1963). These workers observed a 10 percent growth stimulation by supplementing a 30 percent soybean protein diet with 0.5 percent DL-methionine. Addition of 1 percent DL-methionine was required for maximum growth with a 20 percent soybean protein diet.

The sulfur-containing amino acid requirement of the young guinea pig fed a 20 percent soybean protein diet supplemented with adequate methionine and cystine was found to be 0.71 percent of the diet with 0.36 percent L-cystine and 0.35 percent L-methionine present (Reid, 1966). Better growth was obtained with 0.375 percent of L-methionine than with 0.75 percent of the DL form, and growth was poor with addition of 0.375 percent of the D isomer. In contrast to findings in the chick (Fell *et al.*, 1959) and rat (Wretlind and Rose, 1950), D-methionine is not as active as the L isomer in the guinea pig (Reid, 1966).

Between 0.16 and 0.20 percent dietary tryptophan was required by growing guinea pigs (Reid and von Sallmann, 1960). Signs of tryptophan deficiency were produced by feeding a diet with 10 percent each of soy protein and gelatin, an amino acid mixture, and an ample supply of niacin (20 mg/100 g of diet). Young animals fed this purified diet (0.108 percent L-tryptophan) did not grow well, had distended abdomens, alopecia, and cataracts (Reid and von Sallmann, 1960). Adding 0.03 percent L-tryptophan to the diet produced good growth, but complete protection from cataracts was not obtained until 0.1 percent tryptophan was added. Thus, the requirement for tryptophan to prevent eye lesions was considerably greater than the requirement for maximum growth. The qualitative and quantitative requirements of the guinea pig for other amino acids have not yet been determined.

Guinea pigs perform efficiently on natural-ingredient diets with 18–20 percent protein. Where an adequate but not excessive amount of protein is provided, the pregnant guinea pig has less difficulty with elimination of waste products. Pregnant guinea pigs often have a fetal mass equivalent to the nonpregnant weight of the female. Under this condition, the elimination of waste products is difficult. Growing guinea pigs are not subjected to this type of stress, but several studies have shown that high-protein diets are unnecessary (Lister and McCance, 1965; Shelton, 1971; Wheat *et al.*, 1963).

Enwonwu (1973) studied protein malnutrition in guinea pigs fed a purified diet with 3 percent casein. Pair-fed control animals received a natural-ingredient diet. Those animals fed the purified diet rapidly developed clinical signs similar to the kwashiorkor syndrome, including reduced activity, some hair loss, and extensive edema of the face and forelimbs. Protein-deficient guinea pigs had reduced total protein, albumin, and essential amino acids in the plasma and mild fatty livers. Young guinea pigs qualify as suitable models for the study of human protein–calorie malnutrition (Enwonwu, 1973). Bhuyan and Ramalingaswami (1973) fed guinea pigs purified diets containing either 2 or 20 percent casein and observed that a protein deficiency in the animals was accompanied by marked inhibition of local and systemic immune responses to intradermal vaccination (Bacillus Calmette-Guerin).

FAT

The quantitative requirement of the guinea pig for unsaturated fatty acids was determined by feeding purified diets containing various amounts of corn oil, safflower oil, or methyl linoleate (Reid *et al.*, 1964). Results showed that guinea pigs do well over a wide range of fat intake. Corn oil at a concentration of 1 percent (1.89 percent of calories as linoleate) was adequate for growth and prevention of skin changes. Pure methyl linoleate at 0.4 percent (1.31 percent of calories) was required to support maximum weight gains and prevent dermatitis.

Young guinea pigs fed a fat-free purified diet gained 40 percent less body weight than control animals fed a diet containing 7.3 percent corn oil for 28 weeks (Reid, 1954a; Reid and Martin, 1959). Other signs included dermatitis; skin ulcers; fur loss; priapism; relative underdevelopment of spleen, testes, and gallbladder; and enlargement of kidneys, liver, adrenals, and heart. A mild microcytic anemia developed in some guinea pigs. There was also a marked reduction in dienoic fatty acid content of lipids in serum and erythrocytes, and some of the animals died.

FIBERS AND OTHER BULK FORMERS

In their natural habitat, guinea pigs subsist almost entirely on foods of plant origin, which are high in roughage content. Early investigators recognized the importance of bulk in purified diets and showed that finely ground cellulose and gum arabic were good sources of bulk (Booth *et al.*, 1949; Woolley and Sprince, 1945). Other investigators have continued to use ground cellophane and ground cellulose satisfactorily (Heinicke and Elvehjem, 1955; Navia and Lopez, 1973; Reid and Briggs, 1953). Natural-ingredient diets generally contain 9 to 18 percent crude fiber.

MINERALS

Calcium and Phosphorus

There have been few studies of calcium metabolism in the guinea pig, although several reports describe the relationship of calcium to phosphorus, magnesium, and potassium. The requirement for each of these four elements varies with concentration and ratio of the other three. When adequate amounts of phosphorus and magnesium were present, 0.8 to 1.0 percent of dietary calcium appeared to be sufficient

(Morris and O'Dell, 1963; O'Dell *et al.*, 1960). Specific studies of the calcium requirement for guinea pigs are needed.

A form of rickets was produced in young guinea pigs by feeding a purified diet low in calcium (0.028 percent) and vitamin D (Howe *et al.*, 1940). Of the 21 animals that survived up to 60 days, 9 developed rachitic lesions in teeth and epiphyses of ribs and long bones. However, the diet was low in protein (15 percent casein) and there were no control animals, thus making interpretation difficult.

When dietary amounts of phosphorus are not carefully controlled, alterations in acid-base balance and soft tissue calcification occur in guinea pigs. Hogan and Regan (1946) implicated excess phosphorus as a cause of the latter condition. These findings were confirmed when 90 percent of the guinea pigs fed a diet with 0.8 percent calcium and 0.9 percent phosphorus developed soft tissue mineral deposits, whereas the incidence was less than 10 percent when the diet contained only 0.5 percent phosphorus (Hogan *et al.*, 1950). In subsequent experiments, supplemental magnesium and potassium prevented the effects of excess dietary phosphorus, including soft tissue calcification (House and Hogan, 1955).

Magnesium

Clinical signs of deficiency in young guinea pigs fed low-magnesium purified diets include poor body weight gains, hair loss, decreased activity, poor muscular coordination and stiffness of rear limbs, elevated serum phosphorus, and anemia (Maynard *et al.*, 1958; Morris and O'Dell, 1963; O'Dell *et al.*, 1960). Convulsions that characterize magnesium deficiency in some species were uncommon in guinea pigs (Grace and O'Dell, 1970a; O'Dell *et al.*, 1960), but one study reported tetany (Thompson *et al.*, 1964). Gross tissue changes at necropsy were enlarged pale kidneys, white foci and streaks in liver, and, in chronically deficient animals, soft tissue calcification and defective incisors; incisors were darkened, eroded, and soft (Maynard *et al.*, 1958; Morris and O'Dell, 1961; O'Dell *et al.*, 1960). In addition, Grace and O'Dell (1970b) concluded that magnesium deficiency probably affected appetite and/or membrane transport of nutrients.

The magnesium requirement of young guinea pigs is interrelated with the amount of dietary calcium, phosphorus, and potassium. Morris and O'Dell (1963) concluded that an excess of calcium or phosphorus increased independently the minimum magnesium requirement and that the effects were additive. As dietary phosphorus increased from 0.8 to 1.7 percent, the minimum magnesium requirement increased from 0.1 to 0.4 percent. Similarly, as dietary calcium increased from 0.9 to 2.5 percent, the magnesium requirement increased. With growth and hemoglobin concentration as criteria of adequacy, 0.9 percent calcium, 0.6 percent phosphorus, and 0.3 percent magnesium in the diet supported good animal performance.

Magnesium is important in maintenance of electrolyte distribution. In muscle of magnesium-deficient guinea pigs, the extracellular and intracellular magnesium were reduced respectively, to about 20 and 80 percent of control values, while intracellular sodium and water were increased and potassium was decreased (Grace and O'Dell, 1970a). Potassium was noted to have a significant physiological interaction with dietary magnesium. Supplemental dietary potassium for guinea pigs fed magnesium-deficient diets stimulated growth, lowered blood phosphorus, decreased calcium concentration in muscle, extended survival time, and reduced mortality. Apparently, guinea pigs utilize cations rather than ammonia to neutralize and excrete acid in the urine (O'Dell *et al.*, 1956).

Addition of 100 or 200 ppm fluoride to a magnesium-deficient (0.04 percent) guinea pig diet significantly improved growth ($P < 0.05$); increased serum magnesium; reduced incidence of soft tissue calcification; and reduced calcium concentration in kidney, heart, and liver (Pyke *et al.*, 1967). When magnesium was severely limiting (0.01 percent), 200 ppm fluoride was toxic and caused lameness and swollen feet; but adequate magnesium largely overcame the deleterious effect in weanling guinea pigs (O'Dell *et al.*, 1973).

Potassium

The guinea pig's need for a generous supply of cations to offset an inability to conserve fixed base is reported as justification for its high dietary potassium requirement (1.4 percent) (O'Dell *et al.*, 1956). When sufficient quantities of other dietary cations (calcium and magnesium) are present in the diet, the minimum potassium requirement is projected to be much less than 1.4 percent. Mortality was 100 percent within 4 weeks when young guinea pigs were fed a purified diet (30 percent casein) that supplied excess cations, but only 0.1 percent potassium. The requirement for maximum growth under these circumstances was 0.4 to 0.5 percent from potassium acetate (Grace and O'Dell, 1968).

Young guinea pigs fed a potassium-deficient diet had reduced body weight gains, but membrane potentials in striated muscle cells were higher than in control animals (Luderitz *et al.*, 1971). These effects were accompanied by a significant, and apparently quantitative, increase in $(Na^+ + K^+)$ – ATPase activity in heart muscle cells (Bolte *et al.*, 1971; Erdmann *et al.*, 1971).

Manganese

Young female guinea pigs reared on a purified diet (30 percent casein) low in manganese ($<$2–3 ppm) through one or more pregnancies, and their offspring, fed the same diet, were used to study manganese deficiency (Everson, 1968; Everson and Shrader, 1968; Everson *et al.*, 1959). The effects of maternal manganese deficiency were: reduced litter size, abortions or stillbirths, and signs of ataxia, which persisted in animals kept alive for 2–3 months (Everson *et al.*, 1959). Liver manganese was found to be relatively low in newborn guinea pigs, rabbits, and rats (Lorenzen and Smith, 1947); therefore, depletion of tissue manganese should be accomplished readily. Even though no specific effort was directed to determine the minimum manganese requirement, Everson *et al.* (1959) reported a diet to be adequate with the presence of 40 ppm.

Congenital manganese deficiency results in morphologic and metabolic defects in pancreatic islets and in cartilage. The cellular components of the pancreases of guinea pigs born to manganese-deficient mothers exhibited aplasia or marked hypoplasia with fewer but larger islets (Shrader and Everson, 1968). Some of these survived to become adults and, when subjected to glucose tolerance tests, exhibited a diabetic-type response indicative of reduced capacity to utilize glucose. Manganese supplementation (125 ppm for 2 months) completely reversed the reduced glucose utilization (Everson and Shrader, 1968). The pancreases of these manganese-supplemented animals were found to contain increased numbers of islets, which contained more heavily granulated beta cells (Shrader and Everson, 1968).

Manganese is essential in the biosynthesis of acid mucopolysaccharides (AMPS) in cartilage (Leach, 1967; Leach and Muenster, 1962). Congenital skeletal deformities, such as missing or flattened ribs and joint enlargements, have been observed in offspring of manganese-deficient guinea pigs. The AMPS content of rib and epiphyseal cartilage was reduced in deficient animals (Tsai and Everson, 1967). Other studies have produced histochemical evidence that failure of otolith development in the semicircular canals of manganese-deficient guinea pigs is related to a defect in synthesis of AMPS (Shrader and Everson, 1967).

Copper

Everson *et al.* (1967, 1968) induced copper deficiency in the newborn guinea pig by rearing and maintaining females on a copper-deficient diet throughout pregnancy. The copper-deficient offspring grew slowly; some became moribund or died suddenly during the first month postpartum. Intrathoracic or intraabdominal hemorrhage and aortic aneurysms were found in these animals at necropsy. The elastin content of aorta was markedly reduced at 28 days of age. These vascular lesions resemble those in copper-deficient chicks (Carlton and Henderson, 1963; O'Dell *et al.*, 1961) and pigs (Shields *et al.*, 1962). The lesions were explained on the basis of a copper requirement for synthesis of cross-links in elastin (Partridge, 1966). Guinea pigs maintained on the control diet with 6 mg/kg copper appeared normal (Everson *et al.*, 1967, 1968).

Severely affected copper-deficient newborn guinea pigs showed various signs of central nervous system disturbance, including abnormal head movements, ataxia, and body tremors (Everson *et al.*, 1967). At necrospy, some had hemorrhages in the brain, cerebral edema, and agenesis of cerebellar folia. Histological evaluation and chemical analyses indicated a failure in myelination throughout the brain of copper-deficient animals (Everson *et al.*, 1968). These studies showed that, although the fetal and neonatal guinea pig responds somewhat differently to copper deprivation than the lamb (Barlow, 1963), it nevertheless has good potential as a model for nutritionally induced central nervous system disorders.

The dietary level of molybdenum for the guinea pig has a profound effect upon the copper–molybdenum antagonism. Concentrations of molybdenum below 100 mg/kg of diet decreased the copper nutritional status of the animal, and the extent of copper depletion was proportional to the log of molybdenum concentration in the diet. At molybdenum concentrations above 100 mg/kg, an increased tissue copper concentration was accompanied by clinical copper deficiency (Suttle, 1974).

Zinc

Weanling 200-g guinea pigs fed a purified gel diet containing approximately 19 ppm zinc appeared normal and grew well (Navia and Lopez, 1973). Animals receiving a low-zinc diet (1.2 ppm) exhibited decreased zinc content in the plasma and femur compared with similar tissues from those fed a diet containing 164 ppm zinc (McBean *et al.*, 1972). The guinea pig, like the rat, tolerates high amounts of dietary zinc; those fed 2,000 ppm zinc in a gel diet had no ill effects, although body weights after 20 days on this diet were lower than for guinea pigs receiving 75 ppm zinc (Lopez *et al.*, 1970). Their tibia bone ash averaged 1,300 ppm zinc, while that of the control animals was about 400 ppm. Another group receiving 2,000 ppm zinc plus 2 percent KH_2PO_4 averaged 700 ppm zinc in their tibia bone ash.

Guinea pigs under bone fracture stress required a diet with more than 19 ppm to maintain positive zinc balance. Lopez *et al.* (1973) found that, at 8 days postfracture, guinea pigs maintained on a diet containing 19 ppm zinc were in negative zinc balance (−200 percent of intake), which persisted until termination of the experiment 35 days later. Those animals treated similarly, but on a 100-ppm zinc diet, maintained a positive zinc balance.

Chromium

Preston *et al.* (1976) indicated that 0.125, 0.625, and 50 ppm dietary chromium did not affect body weight gain, glucose tolerance, or serum cholesterol in either adults or their offspring. On this unpalatable diet, made with 32 percent Torula yeast to provide 16.8 percent protein, a relatively high mortality rate occurred among animals with the lowest chromium intake. Chromium supplementation appeared beneficial for guinea pig survival during mating, pregnancy, and lactation. Under experimental conditions, while the diet with 0.625 ppm chromium seemed to improve survival rates, the low amount (0.125 ppm) was sufficient to avoid impairment of glucose tolerance. Zimmerman *et al.* (1974) reported that guinea pig insulin has different properties from bovine and rat insulins. The metabolism of chromium in guinea pigs may be affected by this unique type of insulin.

FAT-SOLUBLE VITAMINS

Vitamin A and Carotenoids

Based upon body weight gains, liver storage of vitamin A, and integrity of epithelial and liver cells, 7 mg of vitamin A (retinol) per kilogram of diet meets the requirements of the 250-g growing guinea pig (Gil *et al.*, 1968). This amount is equivalent to 0.2 mg of vitamin A, on the basis of 30 g of food consumed daily, and is in agreement with the findings of Reid and Briggs (1953). Howell *et al.* (1967) reported that a daily intake of 0.7 mg of retinyl acetate per kilogram of body weight was adequate. Data from these three papers support the conclusion that 7 mg/kg of diet or 0.7 mg/kg of body weight provided daily meets the minimum needs of the young, growing guinea pig for vitamin A, but may not provide a safety margin for losses that may be incurred during diet storage. Carotene is utilized by the guinea pig as a precursor of vitamin A (Chevallier and Choron, 1935, 1936; Woytkiw and Esselbaugh, 1951).

Signs of Deficiency Onset of deficiency signs varies widely with age, liver vitamin A concentrations, and stress conditions. Young guinea pigs may develop deficiency signs in 2 weeks, whereas older pigs may require nearly 10 weeks when fed a diet without any vitamin A or provitamin A. The first evidence of vitamin A deficiency is poor growth, then weight loss followed by incrustations of eyelids and severe dermatitis due to bacterial infection (Bentley and Morgan, 1945). Gross pathology studies often reveal accumulation of organic debris in the bile ducts and gallbladder, clouding of the cornea, and xerophthalmia. Often the animals develop pneumonia prior to death. Histologically, epithelia of various organs showed squamous metaplasia and some keratinization (Howell *et al.*, 1967).

Wolbach (1954) described the effects of vitamin A deficiency on teeth of guinea pigs. The primary effect on incisors was mainly on odontogenic epithelium with incomplete differentiation of cells, loss of organization, and formation of defective dentin by atrophic odontoblasts. The incisors had a distinctive appearance characterized by thickened dentin on the labial side and thin dentin on the lingual and lateral sides.

Signs of Excess Excessive amounts of vitamin A given to guinea pigs caused degenerative changes in the cartilagenous epiphyseal plates of long bones (Wolbach, 1947), and there was increased bone resorption interfering with normal remodeling. Teratogenic effects were noted by Robens (1970) when a single oral dose (200,000 USP units per kilogram of body weight) given to pregnant guinea pigs during fetal organogenesis (days 14 to 20) caused soft tissue and skeletal anomalies in the offspring. The most frequent defects recorded were agnathia, synotia, malpositioning of teeth, and microstomia. Administration of the same dose between days 17 and 20 frequently produced changes in the tibias and fibulas, but fetal growth was not affected.

Vitamin D

Quantitative requirement for vitamin D has not been established for guinea pigs, but currently used natural-ingredient and purified diets contain between 1,000 and 2,000 IU/kg of diet. These amounts seem to promote good growth. Vitamin D-dependent intestinal calcium-binding protein has been found throughout the gastrointestinal tract of the guinea pig (Chapman *et al.*, 1977).

Signs of Deficiency Deficiency of vitamin D did not induce rickets in the studies of Randoin and Lecoq (1930) and by Kodicek and Murray (1943). The latter investigators maintained animals in good health for 3 months on a diet deficient in vitamin D, but with a normal calcium to phosphorus ratio. Guinea pigs housed in a darkened room and fed a low-vitamin D purified diet with 0.028 percent calcium and 0.2 percent phosphorus did not grow normally. Typical lesions occurred in the zone of cartilage proliferation at the epiphyseal plate of long bones and ribs. Also, incisors exhibited a high degree of enamel hypoplasia, while enamel and dentin were disorganized and irregular with poor calcification (Howe *et al.*, 1940).

Signs of Excess Acute or chronic vitamin D toxicity has not been reported in the guinea pig, but soft tissue calcification occurs in mineral imbalance (Navia and Hunt, 1976).

Vitamin E

No precise quantitative requirement for vitamin E can be given in spite of guinea pig studies involving vitamin E and related nutrients. The best estimate of a minimum requirement for the growing guinea pig is 1.0 to 1.5 mg/day (Shimotori *et al.*, 1939); reproducing guinea pigs appear to need 3 mg/day (Farmer *et al.*, 1950).

Signs of Deficiency Diet-induced muscular dystrophy of the guinea pig was produced by Goettsch (1930) and Pappenheimer (1930), but the factor responsible for the dystrophic changes was unknown. Madsen *et al.* (1933) continued studying the muscular changes that occurred when cod-liver oil was included in the diet of the guinea pig. The research of Shimotori *et al.* (1939) related a deficiency of vitamin E to the muscular dystrophy reported in earlier studies. An average oral dose of 1.5 mg of α-tocopherol per day provided protection against signs of muscular dystrophy during a 200-day period. Pappenheimer and Goettsch (1941) confirmed the role of vitamin E for maintenance of normal muscle and extended the observations to show the need for vitamin E during pregnancy.

Schottelius *et al.* (1959) reported that vitamin E deficiency in guinea pigs precipitated a decrease in muscle myoglobin concentration. Reduced myoglobin concentration was observed before the appearance of severe tissue lesions or elevated creatine excretion. Supplementation with vitamin E reduced the magnitude of the myoglobin change. Elmadfa and Feldheim (1971) have shown that creatine phosphokinase activity in skeletal muscle of young male guinea pigs was reduced significantly by maintenance on a vitamin E-deficient diet for 2 weeks. The serum creatine phosphokinase activity increased during the same period. At about 4 weeks, creatine excretion in the urine increased, and by the sixth week erythrocyte hemolysis increased ($P < 0.05$), reaching a maximum at 8 weeks. Soon thereafter, the guinea pigs became prostrate with severe body weight loss and degeneration of skeletal muscle. In males, testes atrophied and developed degenerative changes in the seminiferous tubules, with clumping or complete disappearance of spermatozoa and spermatids. Fetal malformations, resorption, and death occurred in pregnant females.

Vitamin K

Guinea pigs fed a special purified diet without vitamin K for 6 weeks did not develop hemorrhages or abnormal clotting times. In view of the active intestinal flora and coprophagic habits, they do not appear to require dietary vitamin K. Their actual nutritional needs for vitamin K and signs of hypovitaminosis remain to be described (Navia and Hunt, 1976).

WATER-SOLUBLE VITAMINS

Thiamin

The thiamin requirement of the young guinea pig is provided by 2 mg/kg of diet (Liu *et al.*, 1967; Reid and Bieri, 1967). No reports are available to support a definite quantitative requirement for gestation and lactation.

Signs of Deficiency Reduced food intake and weight losses, followed by the development of central nervous system disorders, occurred in young, thiamin-deficient guinea pigs. An unsteady gait appeared with some retraction of the head as the condition progressed. Death occurred within 4 weeks (Liu *et al.*, 1967; Reid, 1954b; Reid and Bieri, 1967).

Riboflavin

The limited research by Slanetz (1943), Reid (1954b), and Hara (1960) is inadequate to establish a riboflavin requirement for the guinea pig. The best estimate is 3 mg/kg of diet (Slanetz, 1943).

Signs of Deficiency By feeding young guinea pigs a purified diet deficient in riboflavin, Reid (1954b) found that they exhibited poor growth; rough hair coats; pale feet, nose, and ears; and early death (2 weeks). Later, Hara (1960) described microscopic lesions, such as corneal vascularization, skin atrophy and chromatolysis, and myelin degeneration in the pons and spinal cord. Myocardial alterations included hemorrhage and edema accompanied by vacuolar degeneration and atrophy.

Niacin

Guinea pigs require a dietary intake of niacin (Reid, 1954b). However, because they can produce niacin from tryptophan, the niacin requirement is influenced by quantity and quality of dietary protein, especially tryptophan content and availability. According to Reid (1961), 10 mg of niacin per kilogram of diet was adequate in a purified diet containing 30 percent casein or 20 percent casein supplemented with 1 percent L-arginine.

Signs of Deficiency The most definitive reports on niacin deficiency in the guinea pig are those of Reid (1954b, 1961). When niacin was omitted from a 30 percent casein purified diet, deficiency signs were observed in 3 to 4 weeks. All deficient animals exhibited poor growth; small appetite; pale feet, nose, and ears; drooling, anemia; and a tendency to diarrhea. The animals also had lowered hemoglobin and hematocrit. There were no oral or ocular lesions and no dermatitis.

Vitamin B_6 (Pyridoxine)

Based on weight gain and general appearance of the growing guinea pig, the quantitative requirement is 2 to 3 mg/kg of diet (Reid, 1964).

Signs of Deficiency When fed a 30 percent casein purified diet with no pyridoxine added, 15 of 27 animals lived for 8 weeks (Reid, 1964). The living animals grew slowly, but showed no specific clinical signs of deficiency. Some pyridoxine may have been present in the casein used in the diet.

Folic Acid

The young guinea pig appears to have a very high requirement for folic acid. Current evidence supports a quantitative requirement of 3 to 6 mg/kg of diet (Mannering, 1949; Reid, 1954b; Reid *et al.*, 1956; Woodruff *et al.*, 1953). Guinea pigs practice coprophagy and may obtain folic acid from bacterial synthesis. As the animals mature, less folic acid is required.

Signs of Deficiency Young guinea pigs fed a folic acid-deficient diet grew slowly at the beginning and became weak as diet intake gradually declined. Anemia and leukopenia developed. Hemoglobin and hematocrit values decreased, and the bone marrow became aplastic. Fatty livers and adrenal hemorrhages were prominent at necropsy (Reid, 1954b; Reid *et al.*, 1956; Woodruff *et al.*, 1953).

Pantothenic Acid

The young guinea pig has a high requirement for pantothenic acid—between 15 and 20 mg/kg of dry diet (Reid and Briggs, 1954). The adult requirement has not been established. It is projected to be similar to that of young animals, as nonpregnant or pregnant adults can be depleted rather rapidly (Hurley *et al.*, 1965).

Signs of Deficiency Young guinea pigs fed a purified, pantothenic acid-deficient diet developed signs of deficiency such as decreased growth rate, anorexia, weight loss, rough coat, diarrhea, weakness, and death (Reid and Briggs, 1954). Hair pigmentation was unaffected, and the adrenals were enlarged and sometimes hyperemic or hemorrhagic. Adult animals fed a pantothenic acid-deficient diet died within 10 to 41 days (Hurley *et al.*, 1965). Many of them had adrenal and gastrointestinal hemorrhages.

Biotin

No quantitative requirement for biotin has been demonstrated for normal, healthy guinea pigs. Reid (1954b) observed no significant change in growth of young guinea pigs fed a purified diet with biotin omitted. Feeding a biotin-deficient diet containing raw egg white produced weight loss, alopecia, and depigmentation of the fur (Coots *et al.*, 1959).

Choline

The inclusion of 1.0–1.5 g of choline chloride per kilogram of purified diet supported acceptable growth of young guinea pigs (Reid, 1955). Considerable opportunity remains for elucidation of conflicting reports (Navia and Hunt, 1976) in the literature concerning the success of inducing choline deficiency in the guinea pig.

Signs of Deficiency Choline deficiency has been characterized in very young guinea pigs (Reid, 1954b, 1955). When 2- to 4-week-old guinea pigs were fed a 30 percent casein diet lacking added choline, but adequate in folic acid and vitamin B_{12} (10.0 and 0.04 mg/kg of diet, respectively), poor growth, anemia, and muscular weakness were observed. Some adrenal and subcutaneous hemorrhages occurred, but no renal hemorrhages or marked fatty infiltration of liver were reported.

Inositol

There is no evidence that the guinea pig requires a dietary source of inositol. Reid (1954b) did not observe a significant growth depression when inositol was omitted from a purified diet.

Vitamin B_{12}

There is no unequivocal evidence that the growing guinea pig requires a dietary source of vitamin B_{12} (Reid, 1954b). Guinea pigs may ingest a significant amount of that vitamin by practicing coprophagy.

Ascorbic Acid (Vitamin C)

The ascorbic acid requirement of the guinea pig has been reviewed by Mannering (1949). The daily requirement (in milligrams per animal) varied according to the criterion used to evaluate adequacy: growth, 0.4 to 2.0; macroscopic scurvy, 0.5; microscopic scurvy, 1.3 to 2.5; odontoblast growth, 2; wound healing, 2; bone regeneration, 2; serum phosphatase, 0.23; reproduction, 2 to 5; prolonged survival, 5 or less; and tissue saturation, 25 to 30. Approximately 7 mg of ascorbic acid per kilogram of body weight was adequate to maintain adrenal size and odontoblast height in male guinea pigs ranging in size from 110 to 840 g (Pfander and Mitchell, 1952). Collins and Elvehjem (1958) found 5 mg/kg of body weight sufficient for growth of immature guinea pigs. Daily intakes of 6 to 10 mg will provide adequate amounts for growth and reproduction; normal intake of a diet with 200 mg/kg will fulfill this need. Sorenson *et al.* (1974) reported that guinea pigs conditioned to very high amounts (86 g/kg of diet) of ascorbic acid catabolized radioactive-labeled ascorbate much faster than similar pigs fed a high but more normal amount (2 g/kg of diet). This accelerated catabolism was not reversed by reducing the level to 3 mg/kg for 68 days or even providing a diet devoid of ascorbic acid for 44 days. The stability of ascorbic acid in diets varies with the composition of the diet, storage temperature, and humidity. Approximately one-half of the initial ascorbic acid may be oxidized and lost 90 days after the diet has been mixed. Aqueous solutions may lose vitamin C potency very rapidly.

Signs of Deficiency Early signs of vitamin C deficiency in guinea pigs were reduced diet intake and weight loss, followed by anemia and widespread hemorrhages. Deficient animals were dead within 3 to 4 weeks from the above changes or from secondary bacterial infections to which they are susceptible. Ascorbic acid is essential in hydroxylase reactions for the formation of hydroxyproline and hydroxylysine in the collagen molecule (Stone and Meister, 1962; Udenfriend, 1966). Impaired synthesis of this ubiquitous molecule has many effects on the guinea pig, including enlarged costochondral junctions, disturbed epiphyseal growth centers of long bones, bone loss, altered dentin, and gingivitis. Growth and maintenance of connective tissue in skin, fetal tissues and repairing wounds depend upon a supply of dietary ascorbic acid (Barnes *et al.*, 1969a,b, 1970; Rivers *et al.*, 1970). The characteristic hemorrhages in subcutaneous tissues, joints, skeletal muscle, and intestine of scorbutic guinea pigs result from defective connective tissue. An impaired clotting mechanism, as indicated by increased prothrombin time, also contributes to hemorrhaging in vitamin C deficiency. Navia and Hunt (1976) summarized other major metabolic roles for ascorbic acid in the guinea pig.

UNIDENTIFIED GROWTH FACTORS

Essential dietary factors for the guinea pig have been reasonably well defined except for the precise quantitative needs for a few nutrients. However, the development of the casein purified diet by Reid and Briggs (1953), brought out several reports that suggested that guinea pigs require unrecognized compounds to support maximum growth. Ershoff (1957) reported a growth response in guinea pigs fed desiccated alfalfa mixed with a dried milk ration containing minerals. Reid and Mickelsen (1963) found evidence that replacement of part of the casein in their purified diet with 27 percent alfalfa meal, thereby maintaining a constant protein level, resulted in a significant increase in growth rate. Similar evidence by Lakhanpal *et al.* (1966) associated unidentified growth factor activity with alfalfa meal and other plant sources. Furthermore, guinea pig rations prepared with natural feedstuffs, including alfalfa meal, possess this activity.

Singh *et al.* (1968) attempted to identify or characterize the components of natural products that stimulate the growth rate of guinea pigs fed purified diets. They concluded that cabbage stimulated growth by providing a more nearly continuous supply of ascorbic acid. The active principle appeared to be found in the water-insoluble residue of oven-dried alfalfa, and, according to the authors, it may be metabolically related to ascorbic acid, perhaps through the intestinal flora. In spite of these efforts, no new growth factors have been identified (Singh *et al.*, 1968).

EXAMPLES OF ADEQUATE DIETS

The composition of a natural-ingredient diet and three purified diets that have been used successfully with guinea pigs is shown in Tables 14 and 15, respectively. A summary of nutrients recommended for growing guinea pigs is found in Table 16.

TABLE 14 Composition of the Natural-Ingredient Diet Used for Guinea Pigs at the National Institutes of Health

Ingredient	Percent
Alfalfa meal	38.15
Ground wheat	28.90
Ground oats	17.75
Soybean meal	13.25
Ground limestone	1.10
Iodized salt	0.50
Dicalcium phosphate	0.25
Minerals[a]	0.05
Vitamins[b]	0.05

[a] A mineral mix containing 12 percent manganese from manganous oxide, 10 percent zinc from zinc oxide, 8 percent iron from iron sulfate, 0.8 percent copper from copper sulfate, 0.2 percent iodine from ethylene diamine dihydroiodide, 0.1 percent cobalt from cobalt carbonate, and bentonite as an extender is added at the rate of 0.55 g/kg of diet.

[b] Ascorbic acid is added at the rate of 0.62 g/kg of diet, vitamins A and D at 2000 IU/kg, and vitamin E at 18 mg/kg.

TABLE 15 Examples of Purified Diets Used For Guinea Pigs[a]

Ingredient	Reid and Briggs, 1953 (g/kg)	Everson *et al.*, 1959 (g/kg)	Navia and Lopez, 1973 (g/kg)
Casein	300	—	—
Casein (vitamin free)	—	300	300.0
Cornstarch	200	200	—
Sucrose	103	100	431.4
Glucose	78	106	—
Cellophane	15	—	—
Wood pulp	—	100	—
Cellulose	—	—	130.1
Agar	—	50	20.0
Cottonseed oil	—	50	40.0
Corn oil	73	—	—
DL-methionine	—	—	2.0
L-arginine hydrochloride	3	—	—
Salt mixture	60[b]	60[c]	72.2[d]
Potassium acetate	25	25	—
Magnesium oxide	5	5	—
Vitamin mixture	—[e]	—[f]	3.3[g]
Choline chloride	2	2	1.0
Inositol	2	2	—

[a] See each reference for the special diet preparation.

[b] The salt mixture of Fox and Briggs (1960) is preferred to the one originally used in this diet.

[c] The salt mix contained (g): $CaCO_3$, 300; K_2HPO_4, 325; NaCl, 168; $FeSO_4 \cdot 7H_2O$, 25; $MgSO_4 \cdot 7H_2O$, 28; KI, 0.8; $ZNCO_3$, 0.25; $CuSO_4 \cdot 5H_2O$, 0.3; $MNSO_4$, 2.3.

[d] Mineral ingredients (g/kg of diet): $KC_2O_2H_3$, 27; MgO, 5; $CaCO_3$, 14.50; $CaHPO_4$, 8.30; $MgSO_4$, 0.50; $MgCO_3$, 1; NaCl, 2.80; $Fe(PO_4)$, 1.60; KIO_3, 0.038; $MNSO_4 \cdot H_2O$, 0.80; $ZNSO_4 \cdot 7H_2O$, 0.025; $CuSO_4$, 0.036; $CoCl_2 \cdot 6H_2O$, 0.03; $AlK(SO_4)_2 \cdot 12H_2O$, 0.007; NaF, 0.04; KCl, 4.50; sucrose, 6.024.

[e] Vitamin mixture supplied (mg/kg of diet): thiamin · HCl, 16; riboflavin, 16; niacin, 200; pyridoxine · HCl, 16; Ca pantothenate, 40; folic acid, 10; biotin, 0.6; vitamin B_{12}, 0.04 ascorbic acid, 2000; vitamin A acetate, 6; vitamin D_2 (calciferol), 0.04; α-tocopherol acetate, 50; menadione, 2.

[f] Vitamin mixture supplied (mg/kg of diet): thiamin · HCl, 16; riboflavin, 16; niacin, 200; pyridoxine · HCl, 16; Ca pantothenate, 40; folic acid, 10; biotin, 1; vitamin B_{12}, 0.05; *p*-aminobenzoic acid, 100; 2-methyl-naphthoquinone, 5; α-tocopherol, 100; vitamin A, 6000 IU; vitamin D, 600 IU. Also by oral administration: ascorbic acid equivalent of 15 mg/kg; α-tocopherol of 1.5 mg/day for young animals, and 3.7 mg/day for adults.

[g] Vitamin mixture supplied (g/kg of diet): thiamin, 0.01; riboflavin, 0.01; niacin, 0.05; inositol, 1; Ca pantothenate, 0.03; pyridoxine · HCl, 0.01; folic acid, 0.01; vitamin B_{12} (triturated with mannitol at a concentration of 0.1 percent), 0.03; biotin, 0.0002; ascorbic acid, 2; vitamin A, 28,500 IU; vitamin D_2, 285 IU; DL-α-tocopherol, 0.04; menadione, 0.01.

TABLE 16 Recommended Nutrient Allowances for Growing Guinea Pigs[a]

Nutrient	Amount in Diet	Comments
Protein	18%	Shelton, 1971; Lister and McCance, 1965; Wheat *et al.*, 1963.
Unsaturated fatty acid	<1%	1% corn oil satisfactory; Reid *et al.*, 1964
Digestible energy	3 kcal/g	Estimate; no quantitative data
Fiber	10%	Cellulose and/or materials of low digestibility to supply nonnutritive bulk
Calcium	0.8 to 1.0%	Requirement for any one of these varies depending on dietary concentration of the other three; Morris and O'Dell, 1963
Phosphorus	0.4 to 0.7%	
Magnesium	0.1 to 0.3%	
Potassium	0.5 to 1.4%	
Zinc	20 mg/kg	Lopez *et al.*, 1973
Manganese	40 mg/kg	Everson *et al.*, 1959
Copper	6 mg/kg	Everson *et al.*, 1967, 1968
Iron	50 mg/kg	Estimate; no quantitative data
Iodine	1 mg/kg	Estimate; no quantitative data
Selenium	0.1 mg/kg	Estimated from adequate diets
Chromium	0.6 mg/kg	Preston *et al.*, 1976
Vitamin A	7 mg/kg	Gil *et al.*, 1968; Howell *et al.*, 1967; Reid and Bieri, 1967
Vitamin D	1,000 IU/kg	Adequate; no quantitative data; 1 IU = 0.025 μg cholecalciferol
Vitamin E	50 mg/kg	Shimotori *et al.*, 1939
Vitamin K	5 mg/kg	Hypovitaminosis has not been produced
Vitamin C	200 mg/kg	Mannering, 1949
Thiamin	2 mg/kg	Liu *et al.*, 1967; Reid and Bieri, 1967
Riboflavin	3 mg/kg	Slanetz, 1943
Niacin	10 mg/kg	Reid, 1964
Pyridoxine	3 mg/kg	Reid, 1961
Pantothenic acid	20 mg/kg	Reid and Briggs, 1954; Hurley *et al.*, 1965
Choline	1 g/kg	Reid, 1955; Navia and Lopez, 1973
Folic acid	4 mg/kg	Reid *et al.*, 1956; Woodruff *et al.*, 1953
Biotin	0.3 mg/kg	No requirements established; Reid, 1954b; Coots *et al.*, 1959
Vitamin B_{12}	10μg/kg	No requirement established; Reid, 1954b

[a] These recommended nutrient allowances for the growing guinea pig are intended to provide the minimal requirements. Unfortunately, many of the research reports listed a range of amounts indicating that a precise requirement was not available for many of the nutrients. Additional amounts may be needed for reproducing guinea pigs.

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NUTRIENT REQUIREMENTS OF THE HAMSTER

The hamster most widely used in laboratory studies is the golden or Syrian species, *Mesocricetus auratus*. For hamsters other than the golden, nutritional requirements are virtually unknown. A report on the large European hamster, *Cricetus cricetus*, described stomach contents of animals examined at different seasons of the year (Surdacki, 1964). Nutritional studies with the mouselike Chinese or gray hamster, *Cricetulus griseus*, have focused on diet in relation to diabetes (Boquist and Lernmark, 1969; Gerritsen *et al.*, 1974), but no studies have been made of its general dietary requirements (Moore, 1965; Yerganian, 1967).

Golden hamsters have been assumed to grow satisfactorily when fed diets adequate for rats, but primary references to hamster nutrition are infrequent (NRC, 1972). Knowledge of the nutritional requirements of golden hamsters has been obtained generally through studies oriented to the etiology and/or prevention of diseases such as dental caries, gallstones, and tumors.

The compositions of two purified diets and a natural-ingredient diet that have provided sufficient nutrients for satisfactory growth and diet efficiency for hamsters during the 6 weeks following weaning are given in Table 17.

GROWTH AND REPRODUCTION

An accurate description of the growth of laboratory golden hamsters is difficult. As Granados (1951) indicated, some investigators have reported that females grow more rapidly and reach higher mature weights, while other investigators have found males to be larger than females throughout most of the life span. Body weights up to 12 weeks of age for two strains of animals appear in Table 18. In both strains males were generally larger than females. In Table 19, however, the range of weights and average weights of animals in an outbred stock demonstrate that females may reach greater size than the males at maturity.

Average daily diet intakes for laboratory hamsters were approximately 5-7 g (Arrington *et al.*, 1966), and 5-9 g (Banta *et al.*, 1975).

One commercial producer of hamsters obtained an average litter size of 9.06 pups per litter at weaning. Another producer weaned an average of 7, 9, and 10 pups for first, second, and third litters, respectively. Soderwall *et al.* (1960) reported an average litter size at weaning of 6.9 in 161 pregnancies from animals 1-13 months of age. Poiley (1950) had 10 as the highest monthly average litter size in 1,551 pregnancies.

FAT

Knapka and Judge (1974) found that the optimal dietary concentration of crude fat for growing hamsters is approximately 5 percent. In a 35-day trial with 3, 5, 7, and 9 percent dietary fat levels, hamsters showed gains of approximately 47, 51, 52, and 51 g, respectively. Corresponding diet utilization values of 5.9, 6.0, 6.2, and 6.2 g of diet consumed per gram of gain were obtained. However, mortality percentages of 5.7, 5.7, 18.8, and 13.6 were experienced on the 3, 5, 7, and 9 percent fat diets. It appears that natural-ingredient diets with only 4 percent crude fat are adequate for growth, feed efficiency, and longevity. The diets fed hamsters by Knapka and Judge (1974) contained 21 percent crude protein and 11 percent crude fiber.

Convulsions were produced in hamsters after tube feeding of fats high in saturated fatty acids but not by unsaturated fats (Swank and Nakamura, 1960). Postulated causes of the seizures were a deficiency in cerebral oxygen related to sluggish circulation, or fat-induced changes in electrolyte balance (Swank and Jackson, 1963; Swank and Nakamura, 1960).

Serum lipids in normal and tumor-bearing hamsters show marked differences in percentages of phospholipids and triglycerides (Cox and Gökcen, 1974). Scaly skin, alopecia, and increased production of cerumen have been identified as signs of dietary fat deficiency in hamsters (Christensen and Dam, 1952).

Hamsters show several unusual characteristics in regard to their cholesterol metabolism. Serum cholesterol, which is higher in hamsters than in rats, mice, or guinea pigs (Beher *et al.*, 1963), may be increased by feeding cholesterol (Beher *et al.*, 1963; Cohen *et al.*, 1963), bile salts, or corn oil (Beher

TABLE 17 Diets for Satisfactory Growth and Feed Efficiency in Hamsters

	Purified Diets		Natural-Ingredient Diets	
	Arrington *et al.*, 1966 (% in diet)	Rogers *et al.*, 1974 (% in diet)	Banta *et al.*, 1975 (% in 15% protein diet)	
Casein, vitamin-free	18.0	24.0	Ground wheat	13.47
			Soybean meal	10.77
			Ground corn	8.62
Sucrose	28.0	21.9	Ground oats	7.54
			Brewer's yeast	6.46
Cornstarch[e]	35.5	40.0	Cornstarch[e]	36.67
			Fish meal (herring)	4.85
Cellulose fiber	5.0	5.0	Cellulose[f]	2.04
			Alfalfa meal	2.15
Vegetable oil	6.0	3.0	Soybean oil	4.53
Mineral mix	5.0[a]	5.0[c]	Minerals[g]	1.90
NaCl	0.5	—		
Vitamin mix	2.0[b]	10.99[d]	Vitamins[h]	1.00
Analysis:				
Protein (% kcal/g)	16.1	21.0		15.0
	4.2	—		4.4

[a] USP XIV mixture.

[b] *Vitamin Diet Fortification*, Nutritional Biochemical Corp., Cleveland, Ohio.

[c] Salt mixture, g/kg of diet: NaCl, 5.254; potassium citrate, 11.84; potassium phosphate, 3.867; calcium phosphate, 17.777; magnesium carbonate, 2.044; ferric citrate, 0.800; cupric sulfate, 0.027; manganese sulfate, 0.027; aluminum potassium sulfate, 0.0044; potassium iodide, 0.0022; cobalt chloride, 0.0044; zinc carbonate, 0.0176; sodium fluoride, 0.000044.

[d] Vitamin mixture, g/kg of diet: cornstarch 7.940; choline Cl, 2.000; thiamine HCl, 0.025; riboflavin, 0.015; niacin, 0.100; Ca pantothenate, 0.040; pyridoxine HCl, 0.006; biotin, 0.0006; folic acid, 0.004; menadione, 0.004; vitamin B_{12} (0.1 percent tituration with mannitol), 0.050; inositol, 0.20; para-aminobenzoic acid, 0.006; DL-α-tocopherol (1,1[illegible]0 IU/g), 0.600; vitamin D_2, 2,484.000 IU.

[e] *Clearjel*, National Starch and Chemical Corp., New York.

[f] *Solka-Floc*, The Brown Co., New York.

[g] Each kilogram of premix contained 139.452 g sodium chloride; 389 g potassium phosphate, monobasic; 57.3 g magnesium sulfate (anhydrous); 381.4 g calcium carbonate; 27 g ferrous sulfate; 4.01 g manganese sulfate; 0.79 g cupric sulfate; and 0.023 g colbaltous chloride.

[h] Each kilogram of premix contained 2,000,000 IU vitamin A activity (retinyl palmitate); 200,000 IU vitamin D activity (ergocalciferol); 10,000 IU vitamin E activity (DL-α-tocopheryl acetate); 0.5 g menaquinone; 200 g choline; 10 g *p*-aminobenzoic acid; 10 g myoinositol; 4 g niacin; 4 g calcium D-pantothenate; 0.8 g riboflavin; 0.5 g thiamin-HCl; 0.5 g pyridoxine HCl; 0.2 g folacin; 40 mg biotin; 3 mg vitamin B_{12}; and dextrose to make 1 kg.

TABLE 18 Average Body Weights of Two Representative Strains of Hamsters

	Body Wt. (g) of Hamsters Aged (days)					
	21	28	35	56	70	84
Standard LVG Animals						
Male	35	52	70	102	110	125
Female	35	54	66	97	107	115
	Body Wt. (g) of Hamsters Aged (days)					
	20	30	40	60	70	90
Ela:ENG (SYR) Animals						
Male	36	61	82	102	108	126
Female	38	60	81	100	107	124

TABLE 19 Range of Body Weights and Average Weights of Outbred Golden Hamsters Cr:RHG (SYR)[a]

	Body Wt. (g) of Animals Aged (days)				
	21	28	56	84	168
Range of Weights					
Male	29–51	32–71	86–98	99–109	128–142
Female	30–50	31–69	85–104	103–127	150–167
Average Weights					
Male	40	49	92	104	141
Female	40	44	95	115	158

[a] Poiley, S. M. 1972. Weights of animals raised at the National Cancer Institute.

et al., 1963). Plasma cholesterol concentrations were highest in animals fed diets containing 100 g/kg cellulose and lower in animals fed a diet containing *Fusarium* mold 100–400 g/kg (Owen *et al.*, 1975). Cholesterol was not found in the adrenals of hamsters (Cohen *et al.*, 1963; Schindler and Knigge, 1959). Although only moderately susceptible to atherosclerosis (Rossi *et al.*, 1965), hamsters readily produce both cholesterol and cholesterol-free gallstones.

For more than 20 years investigators in different laboratories have designed diets for production, removal, and prevention of gallstones in hamsters (Bergman and van der Linden, 1974, 1975; Christensen and Dam, 1952; Prange *et al.*, 1975; Robins and Fasulo, 1972).

CARBOHYDRATES

Purified and natural-ingredient diets generally fed to hamsters contain 30–40 percent cornstarch (Arrington *et al.*, 1966; Banta *et al.*, 1975; Rogers *et al.*, 1974). Earlier studies on dietary requirements, dental caries, and gallstones employed diets containing up to 60–65 percent of glucose or sucrose (Granados, 1951; Hamilton and Hogan, 1944; Salley and Bryson, 1957). Ershoff (1956) demonstrated the superiority of cornstarch in hamster diets when he obtained 80–100 percent survival in three experimental groups of animals compared to 0 to 30 percent survival of eight groups fed glucose and sucrose diets. In an investigation (Gustafson *et al.*, 1955) of the cariogenic properties of carbohydrate diets, inclusion of lactose and fructose depressed growth and resulted in high mortality (Table 20).

Campbell and Zinner (1970) demonstrated cariogenic effects with diets that contained 40 percent sucrose. Acceptable growth was obtained with glucose, glucose–fructose, fructose, and sucrose diets, but lactose significantly depressed weight gains. Dietary starch may serve either as a more suitable medium for microflora, or it may provide for a more satisfactory colonic pH (Snog-Kjaer *et al.*, 1963).

Hamster mortality on diets high in purified sugars (60 percent or over), is reduced by replacement of sugar by cornstarch and/or reduction of sucrose to 54 percent and inclusion of 8 percent cellulose (Salley and Bryson, 1957). Addition of 20 percent alfalfa meal to high-sugar diets increased growth and decreased mortality (Ershoff, 1956). Basal diets used in experimental studies with hamsters include cornstarch with 5 to 11 percent cellulose (Arrington *et al.*, 1966; Banta *et al.*, 1975; Rogers *et al.*, 1974).

TABLE 20 Effect on Survival of Various Carbohydrates in Diets Fed Hamsters

Carbohydrate	Percent in Diet	Mortality (%)
Lactose	65	22
Fructose	65	22
Glucose	71	6
Sucrose	62	3
Dextrin	65	1

Arvola and Forsander (1963) reported that in free-choice experiments male hamsters prefer 10 percent ethanol solution to water for drinking. Females, on the other hand, drank slightly more water than ethanol solution and did not clearly discriminate between the two. Additional studies indicated that hamsters might serve as ideal experimental animal models for the study of the effects of alcohol ingestion (Arvola and Forsander, 1961; Carver *et al.*, 1953; Emerson *et al.*, 1952; and Reiter *et al.*, 1974). This general acceptance or preference for alcohol by hamsters may be related to the fact that fermentation takes place in the pregastric pouch (Hoover *et al.*, 1969). Limited quantities of alcohol may actually serve as a natural nutrient for hamsters.

ENERGY

The gross caloric intake of hamsters fed purified diets containing 12, 16, and 20 percent casein was found to be 27–29 kcal per day per 100/g of body weight when they were gaining about 40 to 100 g over a 42-day period (Arrington *et al.*, 1966). Smaller hamsters (45 g) consumed 58 kcal per 100 g of body weight per day, while larger animals (90 g) consumed 28 kcal per 100 g of body weight per day.

The 16 percent casein purified diet (Arrington *et al.*, 1966) had a determined gross energy content of 4.2 kcal/g compared to a calculated density in the natural-ingredient diet (Banta *et al.*, 1975) of 4.4 kcal/g. No studies have been made on the effects of restricted energy intakes on longevity, gestation, or lactation.

Previous reports of the oxygen consumption of the golden hamsters have ranged from 0.93 ml/g/h at 30°C (Adolph and Lawrow, 1951) to 2.9 ml/g/h at 5°C (Lyman, 1948). Simek (1975) reported oxygen consumption of 1.09 ml/g/h at 30°C, 2.24 at 20°C, and 3.12 at 10°C in hamsters on high-protein diets.

PROTEINS AND AMINO ACIDS

Hamsters fed for six weeks after weaning on purified diets with 8, 12, 16, and 20 percent casein showed daily weight gains of approximately 0.58, 1.23, 1.39, and 1.38 g, respectively. The corresponding feed utilization values were 9.5, 5.3, 5.0, and 4.9 g of feed per gram of body weight gain. There was no significant improvement in feed utilization with dietary casein levels above 12 percent. When casein was the dietary protein, the amount needed for maximum hamster growth was about 16 percent (Arrington *et al.*, 1966). In the studies with hamsters fed purified diets (Arrington *et al.*, 1966) and natural-ingredients diets (Banta *et al.*, 1975) the corresponding values were 38 and 34 mg/kcal of gross energy, respectively. Only the 10 percent protein diet fed hamsters by Arrington *et al.* (1966) had a value of 29 mg protein per kilocalorie of energy intake.

Hamsters gained approximately 1.67 g/day for the first 42 days following weaning, but during the next 42-day period the average weight gain was only 0.48 g/day when fed a purified diet (Rogers *et al.*, 1974). Hamsters gained 1.74 g/day when fed a natural-ingredient diet during 42 days following weaning and 0.43 g/day during the subsequent 42 days (Rogers *et al.*, 1974). Hamsters fed natural-ingredient diets having 5, 10, 15, 20, and 25 percent protein had daily gains during 6 weeks after weaning of 0.24, 1.52, 1.53, 1.76, and 1.81 g, respectively. Corresponding feed utilization values were 22.5, 4.5, 4.8, 4.6, and 4.8 g of feed per gram of gain (Banta *et al.*, 1975).

Satisfactory growth and feed efficiency were obtained with purified diets with 16 percent (Arrington *et al.*, 1966) and 24 percent (Rogers *et al.*, 1974) protein, but Banta *et al.* (1975) concluded that a natural-ingredient diet with 15 percent protein was adequate.

Quantitative and qualitative differences in microflora of rat and hamster stomachs were described by Hoover *et al.* (1969). Because hamsters can retain 71 percent of dietary urea nitrogen (Matsumoto, 1955), their utilization of dietary nitrogen more nearly resembles that of ruminants than that of rats.

When fish protein concentrate, soybean meal, and wheat gluten were used to provide protein at a 10 percent level, growth and protein efficiency ratio values were highest for rats fed fish protein concentrate for a 3-week period (Banta *et al.*, 1975). The soybean meal diet allowed greatest growth and protein utilization in hamsters. Daily gains of hamsters fed diets containing wheat gluten, fish protein concentrate, or soybean meal averaged 0.21, 1.08, and 1.44 g, respectively.

Hamsters fed phenylalanine as a supplement to natural-ingredient and purified diets with varying quantities of casein and dextrose showed increases in plasma concentrations of both phenylalanine and tyrosine and changes in the activity of hepatic enzymes, phenylalanine hydroxylase, and transaminase (Horwitz and Waisman, 1966). With protein levels at 16 percent, a casein diet was compared with a soybean diet and a soybean diet with a 0.5 percent DL-methionine supplement. Hamster weight gains averaged 1.14, 1.12, and 1.24 g/day and ratios of 5.4, 5.4, and 5.3 g of diet intake per gram of body weight gain were observed (Arrington *et al.*, 1966). The soybean diet was calculated to contain 0.22 percent methionine, but the 0.5 percent additional methionine had no observed effect on growth. The casein diet contained 0.60 percent methionine. Methionine was apparently not a limiting amino acid in these studies with soybean and casein diets.

The calculated amino acid levels of the purified 16 percent casein diet of Arrington *et al.* (1966), the purified 24 percent casein diet of Rogers *et al.* (1974), and the natural-ingredient 15 percent protein diet of Banta *et al.* (1975) are contrasted with the current NRC rat requirements in Table 21.

TABLE 21 Calculated[a] Amino Acid Content of Diets That Gave Satisfactory Weight Gains in Hamsters for 6 Weeks Following Weaning

Amino Acid[b]	Arrington *et al.*, 1966 (16% protein)	Rogers *et al.*, 1974 (20% protein)	Banta *et al.*, 1975 (15% protein)	NRC Rat Requirement[c]
Arginine	0.76	0.90	1.10	0.6
Cystine	0.07	0.08	0.29	—
Histidine	0.56	0.67	0.40	0.3
Isoleucine	1.27	1.52	0.89	0.5
Leucine	1.91	2.29	1.39	0.75
Lysine	1.55	1.86	1.20	0.7
Methionine	0.60	0.72	0.32	0.6
Phenylalanine	1.02	1.22	0.83	0.8
Threonine	0.84	1.01	0.70	0.5
Tryptophan	0.22	0.27	0.34	0.15
Tyrosine	1.04	1.25	0.57	—
Valine	1.51	1.81	0.91	0.6

[a] Amino acid of diets calculated from amino acid content of various ingredients of diets as given in Table 11, *Nutrient Requirements of Beef Cattle* (NRC, 1976).

[b] Percentage of dry matter.

[c] See p. 23.

MINERALS

The amounts of minerals present in purified (Arrington *et al.*, 1966; Rogers *et al.*, 1974) and natural-ingredient diets (Banta *et al.*, 1975) producing satisfactory weight gains in hamsters for at least 6 weeks following weaning are in Table 22. The present NRC rat requirements are given for comparison. These data and the following discussions of individual nutrient elements should provide guidelines for the mineral requirements of laboratory golden hamsters until future investigations define more closely the animals' needs.

Calcium and Phosphorus

Normal bone formation occurs in hamsters fed diets that contain 0.6 percent calcium and 0.35 percent phosphorus. In the absence of vitamin D, rickets may be produced in hamsters fed 0.47 percent calcium and 0.2 percent phosphorus (Jones, 1945). Old female hamsters (585 days or over) fed diets with 0.4 percent phosphorus and 0.3, 0.5, or 0.7 percent calcium were in positive calcium balance only at the two higher calcium intakes. Young animals (52 days old) retained calcium when fed 0.3, 0.5, and 0.7 percent in the diet. Hamsters retained only 76 to 80 percent of dietary calcium (Kane and McCay, 1947). Calculated calcium concentrations of 0.54 to 0.59 percent in the diets presented in Table 22 were sufficient for satisfactory growth.

Stralfors (1961) obtained a 54 percent decrease in the incidence of dental caries in hamsters when the calcium content of the diet was increased from 0.45 to 0.68 percent.

Copper

Copper sulfate (10–50 ppm in drinking water) reduces caries and alveolar bone loss (Costich, 1955; Hein, 1953). Copper citrate and copper sulfate at high dietary intakes were either teratogenic or toxic for hamster embryos (Ferm and Hanlon, 1974). Purified and natural-ingredient diets (Table 23) with copper varying from approximately 2 to 13 ppm were adequate for satisfactory growth for 6 weeks following weaning.

TABLE 22 Nutrient Element Concentrations in Semipurified and Natural-Ingredient Diets That Give Satisfactory Body Weight Gains During 6 Weeks Following Weaning (mg/kg of diet)

Mineral	Purified Diets		Natural-Ingredient (15% protein) Diet (Banta *et al.*, 1975)		NRC Rat Requirement[b]
	Arrington *et al.*, 1966	Rogers *et al.*, 1974	Present[a]	Added	
Calcium, %	0.59	0.41	0.25	0.29	0.5
Phosphorus, %	0.30	0.39	0.41	0.17	0.4
Magnesium, %	0.09	0.06	0.11	0.02	0.04
Potassium, %	0.82	0.61	0.58	0.21	0.36
Sodium, %	0.15	0.21	0.09	0.10	0.05
Iron, mg/kg	140.0	154.0	50.0	130.0	35.0
Manganese, mg/kg	3.65	9.0	15.9	0.0	50.0
Copper, mg/kg	1.6	7.0	8.8	3.8	5.0
Zinc, mg/kg	—	9.2	9.4	0.0	12.0
Iodine, mg/kg	1.6	1.7	0.02	0.0	0.15
Cobalt, mg/kg	—	1.1	?	0.02	—
Fluoride, mg/kg	—	0.024	—	—	1.0

[a] Calculated from feed composition table values, NRC (1976).

[b] See p. 23.

Fluoride

In hamsters fed a cariogenic diet, the addition of 1.9 mg/kg fluoride to a commercial citrus beverage reduced the incidence of caries by 48–57 percent (Gedalia *et al.*, 1975). The presence of 25 mg/kg of fluoride in the drinking water of hamsters fed cariogenic diet reduced the number of caries 47 percent, whereas 25 mg/kg of both molybdenum and fluoride produced a 67 percent reduction in caries (Stookey and Muhler, 1964). Overall, the inclusion of 1 mg/kg or less of fluoride is recommended for hamster diets.

TABLE 23 Vitamin Content of Purified and Natural-Ingredient Hamster Diets (mg/kg of diet)

Vitamin	Purified Diets		Natural-Ingredient (15% protein) Diet (Banta *et al.*, 1975)	
	Arrington *et al.*, 1966	Rogers *et al.*, 1974	Present[a]	Added
Vitamin A	90.0	2.0	2.8	12.5
Vitamin C	900.0	—	—	—
Vitamin D	5.0	62.1	—	—
Vitamin E	100.0	600.0	9.8	100.0
Vitamin K	45.0	4.0	0.2	5.0
Choline	150.0	2,000.0	1,118.0	2,000.0
p-Aminobenzoic acid	100.0	6.0	—	100.0
Inositol	100.0	200.0	—	100.0
Vitamin B_{12}	0.027	0.05	12.0	30.0
Niacin	90.0	100.0	52.0	40.0
Ca pantothenate	60.0	40.0	14.0	40.0
Riboflavin	20.0	15.0	4.0	8.0
Thiamin	20.0	25.0	8.9	5.0
Pyridoxine	20.0	6.0	5.1	5.0
Folic acid	1.80	4.0	1.1	2.0
Biotin	0.40	0.6	0.5	0.4

[a] Calculated from feed composition table values, NRC (1976).

Iodine

Hamsters fed an iodine-deficient diet for several months developed thyroid hyperplasia and produced an iodinated protein other than thyroglobulin (Follis, 1962). Until more research is done on the iodine requirements of hamsters, the rat requirement of 0.15 mg/kg should be a satisfactory guideline.

Iron

The dietary concentration of iron required for growth and reproduction in laboratory hamsters has not been determined. Rennie *et al.* (1975) described a method using an iron-deficient diet and repeated hemorrhages for producing in hamsters a model for chronic human iron deficiency.

Zinc

Poswillo and Cohen (1971) reported an apparent inhibitory effect of zinc on the induction by DMBA of tumors in the hamster cheek pouch. Edwards *et al.* (1974) were unable to confirm an inhibitory action when zinc-supplemented (100 ppm) drinking water was used.

Hamsters raised on a zinc-deficient diet showed loss of weight, cessation of growth, and disruption of estrous cycle (Scott and Sano, 1975).

TABLE 24 Estimated Nutrient Requirements of Golden Hamsters[a]

Nutrient	Unit	Amount in Diet	Investigators
Protein	%	15.0	Banta *et al.*, 1975
Fat	%	5.0	Knapka and Judge, 1974
Digestible energy	kcal/g	4.2	Arrington *et al.*, 1966
Amino acid[b]			
Arginine	%	0.76	Arrington *et al.*, 1966
Histidine	%	0.40	Banta *et al.*, 1975
Isoleucine	%	0.89	Banta *et al.*, 1975
Leucine	%	1.39	Banta *et al.*, 1975
Lysine	%	1.20	Banta *et al.*, 1975
Methionine	%	0.32	Banta *et al.*, 1975
Phenylalanine	%	0.83	Banta *et al.*, 1975
Threonine	%	0.70	Banta *et al.*, 1975
Tryptophan	%	0.34	Banta *et al.*, 1975
Tyrosine	%	0.57	Banta *et al.*, 1975
Valine	%	0.91	Banta *et al.*, 1975
Minerals			
Calcium	%	0.59	Arrington *et al.*, 1966
Magnesium	%	0.06	Rogers *et al.*, 1974
Phosphorus	%	0.30	Arrington *et al.*, 1966
Potassium	%	0.61	Rogers *et al.*, 1974
Sodium	%	0.15	Arrington *et al.*, 1966
Cobalt	mg/kg	1.1	Rogers *et al.*, 1974
Copper	mg/kg	1.6	Arrington *et al.*, 1966
Fluoride	mg/kg	0.024	Rogers *et al.*, 1974
Iodine	mg/kg	1.6	Arrington *et al.*, 1966
Iron	mg/kg	140.0	Arrington *et al.*, 1966
Manganese	mg/kg	3.65	Arrington *et al.*, 1966
Selenium	mg/kg	0.1	Bieri and Evarts, 1974
Zinc	mg/kg	9.2	Rogers *et al.*, 1974
Vitamins			
A retinyl palmitate	mg/kg	2.0	Rogers *et al.*, 1974
D	IU/kg	2,484.0	Rogers *et al.*, 1974
E	mg/kg	3.0	Bieri and Evarts, 1974
K_1	mg/kg	4.0	Rogers *et al.*, 1974
Biotin	mg/kg	0.6	Rogers *et al.*, 1974
Choline	mg/kg	2,000.0	Rogers *et al.*, 1974
Folic acid	mg/kg	2.0	Cohen *et al.*, 1971
Inositol	mg/kg	100.0	Rogers *et al.*, 1974
Niacin	mg/kg	90.0	Arrington *et al.*, 1966
Pantothenate (Ca)	mg/kg	40.0	Rogers *et al.*, 1974
Riboflavin	mg/kg	15.0	Rogers *et al.*, 1974
Thiamin	mg/kg	20.0	Arrington *et al.*, 1966
Vitamin B_6	mg/kg	6.0	Rogers *et al.*, 1974
Vitamin B_{12}	μg/kg	10.0	Cohen *et al.*, 1967

[a] Estimates based on minimal amounts in diets adequate for growth for 6 weeks following weaning.

[b] Amino acid content of diets calculated from amino acid content of diet ingredients. See Table 21.

Vitamins

A comparison of the vitamin concentrations calculated for three diets that have produced good growth in laboratory hamsters for 6 weeks following weaning is given in Table 23.

FAT-SOLUBLE VITAMINS

Vitamin A

Two mg/kg of retinyl palmitate in a purified diet gave almost as much growth of hamsters as that of a natural-ingredient diet (Rogers *et al.*, 1974). Normal tracheal cell populations were obtained when animals on a vitamin A-deficient diet were supplemented with 200 μg/week of retinyl acetate (Boren *et al.*, 1974). Abnormalities of tracheal epithelium in vitamin A-deficient organ cultures were reversed by 0.25 μg/ml of retinyl acetate in the culture medium (Clamon *et al.*, 1974). Organ cultures of trachea from vitamin A-deficient hamsters were used for assay of eight analogues of the vitamin (Sporn *et al.*, 1975).

Male hamsters fed a vitamin A-deficient diet showed normal postweaning growth and maintenance of weight with intraperitoneal administration of retinyl acetate (0.023 or 0.046 mol/week). The alpha isomer at the same concentrations gave slower weight gains (Clamon *et al.*, 1975).

Signs of Deficiency Retarded growth, weight loss (after 8 weeks), abnormally coarse and sparse hair, xerophthalmia (from the fourth to seventh weeks), and hemorrhages from external genitalia and anal regions are characteristic of hypovitaminosis A (Hirschi, 1950; Salley and Bryson, 1957). A decrease in the number of ciliated cells per 100 μg of tracheal epithelium is indicative of vitamin A deficiency (Boren *et al.*, 1974).

Signs of Excess Hamsters fed a vitamin A-deficient diet with supplements of 5 mg retinyl acetate biweekly, or 5 mg retinoic acid 5 days a week for 2 weeks, showed ear cartilage degeneration. Biweekly oral doses of 2 or 5 mg of retinyl acetate in addition to a natural-ingredient diet containing 3.6 μg/g of vitamin A produced a hypervitamin A state (Boren *et al.*, 1974). The method of administration of vitamin A may have been responsible for the toxicity observed since intraperitoneally administered vitamin A equivalent to approximately 6 mg/kg of diet was readily utilized by hamsters (Clamon *et al.*, 1975).

Vitamin A toxicity with weight loss, lethargy, and rough hair coat was observed with intragastric administration of 33 mg/kg of body weight retinyl acetate per week (Smith *et al.*, 1975a,b). Large doses of vitamin A inhibited induction of tumors of the forestomach and cervix by carcinogenic hydrocarbons (Chu and Malmgren, 1965).

Hypervitaminosis A is teratogenic in golden hamsters, leading to derangement and collapse of mesodermal tissue, notochordal alterations, and in some instances failure of closure of developing neural tissues (Marin-Padilla, 1966; Marin-Padilla and Ferm, 1965).

Vitamin D

Hamsters do not require dietary vitamin D for prevention of rickets when the dietary calcium to phosphorus ratio is about 2:1 and calcium is at 0.6 percent (Jones, 1945). Yet 2,000–2,500 IU/kg are usually included in experimental diets (NRC, 1972; Rogers *et al.*, 1974). Rickets may be induced in hamsters in the absence of vitamin D, provided that dietary calcium is 0.4 percent and phosphorus 0.02 percent (Jones, 1945).

Cheek pouch carcinomas have been suppressed by the use of vitamin D_2 and D_3 (Rubin and Levij, 1973).

Vitamin E

Natural-ingredient and purified diets that contain 100 or 600 mg/kg of vitamin E support satisfactory growth and development in weanling hamsters (Arrington *et al.*, 1966; Rogers *et al.*, 1974).

Five daily oral doses of 4 mg of α-tocopherol caused recovery and regeneration of cheek pouch skeletal muscle damaged by vitamin E deficiency (West and Mason, 1958). Testicular degeneration resulting from vitamin E deficiency was avoided when animals were fed diets containing 1 mg/kg of α-tocopheryl acetate or 24 mg/kg of γ-tocopheryl acetate and 0.1 mg/kg of selenium as sodium selenite (Bieri and Evarts, 1974). Daily oral supplements of 10 mg of D-α-tocopheryl acetate or 25 mg of D-α-tocopheryl hydroquinone increased testis weights and induced repair of germinal epithelium in vitamin E-deficient hamsters (Mauer and Mason, 1975).

Vitamin E-deficient hamsters demonstrate depressed growth and reversible muscle degeneration (Houchin, 1942; West and Mason, 1958), which may be observed in living striated muscle fibers (West, 1958). Vitamin E deficiency decreases fertility in older females (Soderwall and Smith, 1962) and induces reversible testicular injury in males (Mason and Mauer, 1975).

Vitamin K

Hypoprothrombinemia can be produced in adult male hamsters either by feeding a vitamin K-deficient diet or by treatment with vitamin K antagonists, Warfarin or 2-chloro, 3-phytyl,1,4-naphtho-quinone (chloro-K) (Shah and Suttie, 1975). An intramuscular dose of vitamin K at 10 mg/kg of body weight restored to normal the prothrombin levels of about half of the deficient animals. Hamsters are more sensitive to chloro-K and more resistant to Warfarin than rats (Shah and Suttie, 1975).

WATER-SOLUBLE VITAMINS

Ascorbic Acid

Hamsters may not require a dietary source of ascorbic acid. However, this assumption is based on an experiment that involved only 20 male animals (10 experimental and 10 control) fed a diet with or without 7 mg/kg of ascorbic acid with 20 percent casein and more than 16 percent crude fat, which gave a weight gain of 1.07 g/day (Clausen and Clark, 1943). Hamsters gained 1.77 g/day on a 20 percent protein natural-ingredient diet (Banta *et al.*, 1975).

A reinvestigation of the ascorbic acid requirement for hamsters may be desirable in the light of interest in possible interrelationships between carcinogenesis and dietary ingredients such as vitamin A (Rogers *et al.*, 1974; Smith *et al.*, 1975a,b), vitamin D (Rubin and Levij, 1973), and thiamin (Salley *et al.*, 1962).

Biotin

Satisfactory natural-ingredient and purified diets provide 0.4–0.9 mg/kg of biotin (Arrington *et al.*, 1966; Banta *et al.*, 1975; Rogers *et al.*, 1974). Injection of 4 μg/day of biotin reverses signs of a deficiency syndrome (Rauch and Nutting, 1958). If one assumes that hamsters consumed 6 g of diet per day, this concentration of biotin would be equivalent to approximately 0.7 mg/kg of diet, which is within the range of biotin provided by satisfactory diets.

The use of a special diet that contained 40 percent egg white and 0.5 percent sulfaguanidine resulted in biotin deficiency with reduced weight gain, achromotrichia, nervousness, jerky movements, abnormal posture, and finally complete alopecia (Rauch and Nutting, 1958).

Choline

Hamsters show poor appetite, reduced growth, and fatty livers on a peanut meal diet deficient in choline (Handler and Bernheim, 1949). Purified diets provide 1 to 2 g/kg of choline chloride (Rogers *et al.*, 1974) or 1.8 g/kg of choline bitartrate (Cohen *et al.*, 1971). The natural-ingredient diet of Banta *et al.* (1975) contained approximately 3 g/kg (see Table 23), while the purified diet of Arrington *et al.* (1966) contained 0.15 g/kg.

Folic Acid

Hamsters were protected from development of a folic acid deficiency by the inclusion of 2 mg of folic acid per kilogram of diet (Cohen *et al.*, 1971). This is within the range of folic acid concentrations (1.8–4.0 mg/kg) in the purified and natural-ingredient diets of Table 23.

Anemia, increased urinary aminoimidazolecarboxamide and formiminoglutamic acid (FIGM), reduction of blood and liver folates, and increased vitamin B_{12} and ascorbic acid in the liver are characteristic signs of folic acid deficiency. Hemoglobin, hematocrit, and red cell counts tend to be lower in females than in males on diets including 2 mg of folic acid per kilogram of diet. Hamsters resemble guinea pigs rather than rats in their ability to develop folate deficiency without the use of intestinal antiseptic or folate antagonist (Cohen *et al.*, 1971).

Riboflavin

Purified and natural-ingredient diets containing 12–20 mg/kg of riboflavin give satisfactory growth in hamsters

(Arrington *et al.*, 1966; Banta *et al.*, 1975; Rogers *et al.*, 1974).

In the absence of dietary riboflavin, food and water intake was reduced and the animals were stunted and inactive, with dull coats. Deficiency signs did not occur in animals fed 20 mg/kg of diet (Smith and Reynolds, 1961).

Thiamin

Satisfactory purified and natural-ingredient diets (see Tables 17 and 23) provided 14–25 mg/kg of thiamin. Average serum thiamin was 24.3 μg/100 ml when dietary thiamin was 8.0 mg/kg, but serum concentration was reduced to 9.2 μg/100 ml when dietary thiamin was 4 mg/kg. This lower thiamin intake was associated with chronically deficient animals with depressed growth and more rapid induction of tumors after applications of a chemical carcinogen (Salley *et al.*, 1962).

Vitamin B_{12}

Urinary excretion of methylmalonic acid (MMA) and formiminoglutamic acid (FIGM) was observed, and serum and liver glutathione were increased in animals on vitamin B_{12}-deficient diets (Cohen *et al.*, 1967). These vitamin B_{12}-deficiency signs were reversed in deficient animals after 5 weeks on a diet containing 10 μg/kg of vitamin B_{12} (Cohen *et al.*, 1967). But deficiency of vitamin B_{12} had no apparent effect on body weight, red blood cell count, hematocrit, or hemoglobin (Cohen *et al.*, 1967; Scheid *et al.*, 1950). Feeding a vitamin B_{12}-deficient diet reduced feed efficiency (Scheid *et al.*, 1950).

Pyridoxine

Diets giving satisfactory growth contained 6–20 mg/kg of pyridoxine (Arrington *et al.*, 1966; Rogers *et al.*, 1974).

Loss of appetite is an early and constant result of pyridoxine deprivation. Decreased water intake and urinary output have been observed, as has priapism. An increase in urinary xanthurenic acid is associated with pyridoxine deficiency in hamsters. Although acrodynia is characteristic of pyridoxine deficiency in rats, it was not found in hamsters. Possibly the presence of corn oil in addition to hydrogenated fat in that experimental diet may have prevented the development of dermatitis. The fur of pyridoxine-deprived animals had an unkempt appearance, and crusted lesions were occasionally observed on lips and mouth. Decreased food intake may be a contributory factor in the arrested development and emaciation associated with pyridoxine deficiency. Atrophy of lymphoid tissue, particularly in the thymus, is an outstanding pathological change (Schwartzman and Strauss, 1949).

WATER

An early estimate suggested that hamsters require 10 ml/day (Bruce, 1950) of drinking water; adult males consumed 8.23 ± 0.45 ml and adult females 8.67 ± 0.22 ml (Arvola and Forsander, 1963). The amount of water required obviously depends on the moisture content of ingested food (Knapka and Judge, 1974; Magalhaes, 1968; Rogers *et al.*, 1974; Whitney, 1965) and the type of watering device (Keyes, 1953). It is imperative that nursing young have access to drinking water at all times (Whitney, 1965).

Hamsters, originally residents of hot and arid regions, have developed behavioral patterns as a means of survival at high temperatures (Rodland and Hainsworth, 1974; Schmidt-Nielsen, 1964). During heat stress hamsters mobilize large amounts of water that is secreted as saliva that evaporates from the body surface and aids in maintenance of heat balance at high temperatures. At 40°C male hamsters maintained their temperature below 43.5° for 2–3 hours by the evaporation of an average of 10 mg of water per gram per hour (Rodland and Hainsworth, 1974).

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NUTRIENT REQUIREMENTS OF THE VOLE

Unique characteristics of many animal species have prompted their use for specific investigational purposes. Microtines, especially those designated as voles, including *Microtus pennsylvanicus*, *M. montanus*, *M. californicus*, *M. agresta*, *M. ochrogaster*, and *M. arvalis*, are such animals. Originally captured from fields where they consume a wide range of plant and some animal materials, voles are now maintained in colonies where they have found special usefulness for studies in several disciplines. Subject areas of investigation and references using voles, follow:

1. Nutritive value of forages and cereals (Brinkman *et al.*, 1974; Elliott, 1963, 1973; Keys and Van Soest, 1970; Lynch and Keys, 1968; Markarian and Elliott, 1968; Martinet and Meunier, 1969; Schillinger and Elliott, 1966; Shenk, 1976; Shenk *et al.*, 1971, 1974; Splitter and Shipe, 1976).
2. Antigrowth factors present in natural ingredients (Barnes *et al.*, 1974; Gustine *et al.*, 1974; Kendall and Leath, 1976; Kendall and Sherwood, 1975; Knoblauch *et al.*, 1977; Leath *et al.*, 1974; Marcarian *et al.*, 1968; Negus and Pinter, 1966; Shenk, 1976; Shenk *et al.*, 1974).
3. Energy metabolism (Ashman and Seed, 1973; Ashman *et al.*, 1976; Caillol and Martinet, 1976; Cowan *et al.*, 1968; Drodz, 1968; Drodz *et al.*, 1972; Lee and Horvath, 1969; McManus, 1974; Packard, 1968; Shenk *et al.*, 1970).
4. Comparative responses to those of other animals (Brinkman *et al.*, 1974; Kendall and Sherwood, 1975; Keys and Van Soest, 1970; Shenk, 1976; Shenk *et al.*, 1975).
5. Hormone and reproductive activities (Berger and Negus, 1974; Dobrowolska *et al.*, 1976; Evans, 1973; Martinet and Meunier, 1969, 1975; Negus and Berger, 1971; Pinter, 1968; Pinter and Negus, 1965; Richmond and Conaway, 1969).
6. Response to disease (Ackerman and Seed, 1976; Newport *et al.*, 1977; Stibbs and Seed, 1976).
7. Genetics (Gill, 1976; Richmond and Conaway, 1969).
8. Behavior in natural or domestic environments (Evans, 1973; Hansson, 1971; Kendall and Leath, 1976; Krebs *et al.*, 1973; Martinet and Daketse, 1976).
9. Environmental pollutants and photoperiod responses (Ashman *et al.*, 1976; Caillol and Martinet, 1976; Martinet and Daketse, 1976; Martinet and Meunier, 1969, 1975; Negus and Berger, 1971; Negus and Pinter, 1966; Pinter, 1968; Pinter and Negus, 1965; Williams *et al.*, 1977).
10. Voles are an animal model for plant breeders, because small quantities of test materials can be bioassayed using a 6-day growth trial of weanling voles (Brinkman, *et al.*, 1974; Schillinger and Elliott, 1966; Shenk, 1976; Shenk and Barnes, 1974; Shenk and Elliott, 1969; Splitter and Shipe, 1976).

GENERAL COLONY PROCEDURES OR MANAGEMENT

Laboratory voles have little or no odor, a high reproductive rate, excellent postoperative survival, and are easily handled and maintained. Successful feeding and housing procedures have been published (Berger and Negus, 1974; Drodz, 1968; Elliott, 1963; Lee and Horvath, 1969; Pinter, 1968; Richmond and Conaway, 1969; Russo *et al.*, 1976; Shenk, 1976). Cages and bedding of various types have been suitable. Light cycles of about 15 to 18 hours were apparently more satisfactory than days with light of 6 to 10 hours. Yet the colony maintained by Elliott for more than 17 years has been kept with constant 24-hour exposure to incandescent light (Caillol and Martinet, 1976; Elliott, 1963; Martinet and Meunier, 1969, 1975; Pinter, 1968; Pinter and Negus, 1965).

Room temperatures of 18° to 22°C appear desirable, but temperatures of 10° to 25°C have been successful. The thermoneutral zone was 25° to 30°C and below 25°C oxygen consumption increased as an inverse linear function of ambient temperature (McManus, 1974; Packard, 1968). Death of young between birth and 1 month is much greater at 5°C than at 22° or 31°C (Martinet and Daketse, 1976). Housing at a relative humidity of about 65 to 75 percent is desirable.

Voles are usually kept in solid-bottom cages with nesting material in harems of two to four females from which obviously pregnant females should be separated. Successful matings occur at about 49 days of age, with a 21.5-day average gestation period. Usually four to six young are born with an average birth weight of 2.9 g, depending on age of the female. Young begin eating dry diet at about 12 days of age and usually are weaned at 14 to 18 days of age when they weigh 12 to 14 g. Postpartum estrus and matings occur. When kept in harems, about 60 percent of the females can have litters every 23 days (Elliott, 1963; Lee and Horvath, 1969; Pinter and Negus, 1965; Richmond and Conaway, 1969). Reproduction and estrus activity have been modified by diet and environmental temperature (Berger and Negus, 1974; Martinet and Daketse, 1976; Martinet and Meunier, 1969; Negus and Berger, 1971; Negus and Pinter, 1966; Pinter and Negus, 1965).

GENERAL GROWTH, DEVELOPMENT, AND DIET

Adults have an average weight of about 41 g. The female reproductive rate decreases after 6 months, but reproductive life may continue until 9 to 12 months. Weanlings are capable of gaining at least 1 g/day with growth rate dependent on digestible energy content of the diet. Daily feed consumption is 0.2 to 0.3 g per gram of body weight. Successful diets include alfalfa pellets plus salt (Lee and Horvath, 1969), cereal and nuts (Drodz, 1968), natural-ingredient rodent or rabbit feed with or without added green feeds (McManus, 1974; Packard, 1968; Richmond and Conaway, 1969). Cereal plus protein and mineral supplements with green feeds has been the most successful (Elliott, 1963; Negus and Pinter, 1966; Pinter, 1968). Voles are incapable of consuming hard pelleted diets (Elliott, 1963; Shenk, 1976; Shenk *et al.*, 1970, 1971). Water requirement depends on many environmental variables, but should be allowed *ad libitum* (McManus, 1974).

Passage of food through the digestive tract is rapid. Some diet residues are detected in feces within 1 hour. Complete passage of added chromic oxide occurs by 12 hours, while complete passage of green forage occurs at about 17 hours and cereals at about 40 to 48 hours (Kostelecka-Myrcha and Myrcha, 1964; Lee and Horvath, 1969). A high death rate occurs when food and/or water are absent for 24 hours.

Voles are sensitive to trypsin inhibitors in natural ingredients. Investigations with triticale indicated that 75 percent of the variation in protein quality indices from vole growth was due to differences in trypsin inhibitor in the triticale samples. With voles and other minimally defined species, investigators should be aware of all factors that may influence responses.

Voles show a well-defined diurnal maximum of hepatic glucose-6-phosphatase, fructose-1,6-diphosphatase activity, and glycogen content (Ashman and Seed, 1973; Ashman *et al.*, 1976).

ENERGY AND GROWTH

Limited data exist on specific nutrient requirements for voles. Energy digestibility of natural-ingredient diets is 88 to 92 percent, and energy in urine varies from 1 to 7 percent of gross dietary energy (Drodz, 1968; Drodz *et al.*, 1972; Elliott, 1963; Hansson, 1971). Energy or dry matter digestibility decreases as the proportion of forage or cellulose in the diet increases. Digestibility was 70 percent for a diet containing 27 percent fiber, and it may be as low as 50 percent for an all-grass diet. Digestibility of hemicellulose by voles exceeds that for cellulose (Drodz, 1968; Hansson, 1971; Keys and Van Soest, 1970; Shenk *et al.*, 1971). On the same diet, voles digest more plant cell wall and fiber than do rats but less than do ruminants or swine. Energy digestibility of high-quality forages by voles was 80 to 96 percent that of ruminants. Voles digested 30 percent of alfalfa or grass fibers, but little if any cellulose from "alphacell" (Cowan *et al.*, 1968; Keys and Van Soest, 1970; Shenk *et al.*, 1970, 1971). The cecum contains considerable quantities of volatile fatty acids when voles consume fiber. Lactic acid fermentation occurs in the stomach (Lee and Horvath, 1969; McBee, 1970).

Weanling vole growth attained a maximum of 0.9 g/day or more when diets contained 11 to 24 percent casein, 28 to 53 percent carbohydrate, and 25 to 53 percent cellulose fiber. Deviations from this reduced gain. Voles made rapid adjustments in intake when fed dietary combinations of varying protein, carbohydrate, and fiber levels. With these diets, daily gain (in grams per day) was $2.50 - 1.54X_1 - 0.038/X_1 - 1.39X_2 - 0.204/X_2$, where X_1 is casein in diet and X_2 is carbohydrate plus oil proportions in the diet as decimal fractions (Shenk *et al.*, 1970). A different nonlinear mathematical model estimating weight gain (Y) from intake using 84 diets was developed. It is:

$$Y = \left[\frac{3.10}{1 + e^{0.43\,(\text{DEI} - 8.2)}}\right] - 2.0,$$

where DEI is digestible energy intake in kilocalories per day. This relationship also satisfactorily explained relationships between gain and intake when all published data were combined (Shenk, 1976). Others used a linear relationship to estimate intake from body weight up to 70 days of age. This equation was daily kilocalorie metabolizable energy = $1.52W^{0.63}$, where weight was in grams (Drodz *et al.*, 1972). When comparing growth rates from diets containing variable amounts of fiber and a short growth period (6 days), gut fill is sufficiently large that some correction must be made. Diets containing more than 85 percent cereal give increased intake and growth when about 18 percent cellulose is added to the diet. This amount of fiber also was necessary for maximum gain (Shenk, 1976). Energy and fiber in satisfactory diets are given in Table 25.

Maintenance requirement for voles has been estimated at 0.512 kcal metabolizable energy per gram of body weight per day. Voles gaining 1 g/day will consume 15 kcal of digestible energy per day. Lower intakes cause slower growth rates and an intake of 9 kcal produces no growth (Drodz, 1968; Hansson, 1971; Shenk, 1976; Shenk *et al.*, 1970). Standard metabolic rates measured from oxygen consumption for voles and microtine animals have been much greater than those predicted for other animals of that size based on

TABLE 25 Suggested Desirable Dietary Nutrient Amounts and/or Concentrations for Voles[a]

Nutrient	Maximum Desired Diet Concentration	
	Maintenance	Maximum Gain
Energy (kcal DE/day)[b]	9.0	15.0
Corn Oil (%)	1.0	1.0
Protein[c] (%)	8.0	13.0
Lysine (%)	0.17	0.77
Methionine (%)	0.09	0.42
Tryptophan (%)	0.03	0.12
Cell walls (NDF)[d] (%)	52.0	20.0
Lignocellulose (ADF)[e] (%)	33.0	8.0
NDFS[d] × ADFS[e] (%)	35.0	71.0

[a] Adapted from data in references by Shenk (1976) and Lynch and Keys (1968).

[b] DE = digestible energy, daily intake.

[c] Protein as casein or amino acids. Less available protein should be increased accordingly.

[d] NDF = neutral detergent fiber and NDFS = solubles from neutral detergent fiber determination by difference.

[e] ADF = acid detergent fiber insoluble residue and ADFS = solubles from ADF determination by difference.

interspecies formulas relating metabolism to body weight (Drodz, 1968; Drodz *et al.*, 1972; McManus, 1974; Packard, 1968). Calories gained were only 4.7 percent of calories consumed, which is lower than for growing domestic animals (Drodz *et al.*, 1972). Oxygen consumption, diet intake, and body weight gain are greater at 5° to 10°C than at 25° to 30°C. However, efficiency of gain is less at the lower temperature (Caillol and Martinet, 1976; McManus, 1974).

PROTEIN AND AMINO ACIDS

Protein concentration of 13 to 14 percent (85 percent dry matter basis) resulted in maximum weight gain for weanling voles (Lynch and Keys, 1968; Shenk, 1976; Shenk *et al.*, 1970). Relationships between dietary amino acids and plasma amino acids have not been determined other than finding high plasma concentrations of dicarboxylic acids and low concentrations of histidine when compared to rat plasma (Alieva and Aliev, 1973). Free amino acids in serum were decreased after infection (Newport *et al.*, 1977). Purified amino acid diets indicated maximum body weight gain at a dietary protein concentration of 13 percent or above, with tryptophan, methionine, and lysine concentrations in the protein of 0.9, 3.2, and 5.9 percent, respectively (Shenk, 1976). Crude protein and other nutrient contents in diets found to be satisfactory are listed in Table 25.

VITAMINS AND MINERALS

Data on vitamin and mineral requirements by voles are lacking. Growing voles had a high mortality rate when fed diets containing certain alfalfa strains, and this high mortality rate was prevented by addition of a vitamin supplement to the diet. Niacin alone was effective in certain instances (Elliott, 1963; Schillinger and Elliott, 1966). Purified diets and most natural-ingredient diets used have included 1 to 2 percent of a diluted vitamin mixture and 2 to 3 percent of a mineral mixture (Keys and Van Soest, 1970; Shenk *et al.*, 1970, 1971, 1974, 1975). An estimate of vitamin and mineral requirements based on the amounts fed is not warranted.

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NUTRIENT REQUIREMENTS OF FISHES

INTRODUCTION

The use of fishes in research is expanding because studies with these animals yield unique insights into biochemical, physiological, and disease phenomena. Laboratory researchers have found a biological system in fishes that is comparable to higher animals and, for some phases of research, biological systems that are less complex than those of the higher vertebrates. Investigators who use fishes as experimental animals must maintain them in a well-nourished condition to be assured of valid results (Ketola, 1975a; Klontz and Smith, 1968; Neuhaus and Halver, 1969; Nigrelli, 1953).

There are approximately 30,000 species of fishes, which is more than all other vertebrate species combined. Each taxonomic or ecological group of fishes may have distinct environmental requirements for water quality, temperature, osmolality, pH, dissolved gases, and other factors (Norman and Greenwood, 1963; Lagler *et al.*, 1962). A broad choice of fish species is available to fit into intended research. Certain relatively well-standardized fishes have been developed—coldwater fishes, typified by trout and salmon; coolwater fishes, such as the fathead minnow and bluegill; and warmwater fishes, like channel catfish, guppies, or tilapia (Benoit, 1968; Brauhn and Schoettger, 1975; NRC, 1973; Shelbourne, 1971; Umbreit and Ordal, 1972).

Maintenance of appropriate husbandry conditions must be met for each species to survive (Bardach *et al.*, 1972; Buterbaugh and Willoughby, 1967; Hunn *et al.*, 1968). Alteration of environmental conditions from that which is suitable for each fish species adds a variable to research that usually cannot be measured directly and may lead to erroneous conclusions (Klontz and Smith, 1968; Richardson, 1972; Spotte, 1970). Therefore, facilities used for holding experimental fishes must receive as much attention as the selection of a fish species for the intended research (Cullum and Justus, 1973).

Fishes are subject to environmental- and nutritional-related diseases. Little is known about diseases caused solely by malnutrition. Amino acid and vitamin deficiencies or management stresses such as handling, drug treatments, unfamiliar surroundings, and water temperature fluctuations of more than 10°C may predispose fishes to bacterial, fungal, and protozoal infections (Rumsey and Ketola, 1975; Snieszko, 1974). Minimizing these stresses will ensure more valid results when carefully controlled research is being performed.

Knowledge of nutritional requirements of many of the fishes is limited, but with others, such as the rainbow trout and the channel catfish (NRC, 1977, 1973), knowledge has advanced to a level at which scientific diet formulation is possible (Phillips, 1970; Post, 1972). The following is designed as a guide for maintaining the animals in an optimum state of nutrition to obtain desirable performance.

Practical nutrition of laboratory fishes requires a general understanding of their nutrient requirements (Hashimoto, 1972; Hastings, 1972; Lewis *et al.*, 1969; Peterson *et al.*, 1967; Shell, 1966). The few fishes for which knowledge of nutrient requirements has been set down in specific terms have received enough attention to make possible the compounding of purified diets (Arai *et al.*, 1971; Coates and Halver, 1958; Halver, 1957; Wolf, 1951). Most of the other fishes used as experimental animals receive diets based on empirical observations and employ practical, natural-ingredient diets with vitamin and mineral supplements (Bondy *et al.*, 1957; Crawford *et al.*, 1973; Deuel *et al.*, 1952; Huet, 1973; Poston, 1974). These latter diets may or may not contain all nutritional requirements for survival, growth, health, or reproduction (Gaudet, 1970).

The proper feeding of laboratory fishes should be a necessary part of the experimental protocol of research in which fishes are to be used. The diet of each fish species must provide all essential nutrients without harmful excess of any component.

ENERGY AND METABOLISM

The energy requirements of fishes can be divided into the energy required for maintenance, growth, and reproduction. The maintenance requirement must be satisfied before growth is achieved. Energy for maintenance is further divided into energy for basal metabolism and energy for voluntary activity (Winberg, 1956). Separation of the two components is difficult under most practical conditions, therefore, the term "standard" or "resting" metabolism is usually used with fishes (NRC, 1973). The conditions under which the standard metabolic rate was determined must be carefully defined.

Several factors cause variation in standard metabolic rate in fishes. The temperature of the water and the resulting body temperature of the fish have a great effect (Clausen, 1933; Podoliak, 1961). Each fish species seems to have a preferred temperature at which metabolic processes are optimal (Winberg, 1956). There are species differences in the ability to adapt to temperatures above and below the preferred temperature. The size of the fish also has an effect on metabolic rate. Large fishes of the same species have lower metabolic rates per unit body weight than do small fishes. The magnitude of the change in metabolic rate also varies with oxygen and carbon dioxide content of the water, availability of food, accumulated waste products, diurnal fluctuations, and other environmental factors (NRC, 1973).

The maintenance energy requirement of fishes can be increased by activity. Fishes forced to swim vigorously against a current, or to maintain a desired position in the tank, have high metabolic rates and high maintenance energy requirements. Active fishes fed a fixed ration have less energy remaining for growth than relatively inactive fishes (Hoar and Randall, 1969–1971; NRC, 1973; Winberg, 1956).

Fats, carbohydrates, and proteins are all used as energy sources, and the availability of the energy in these components is dependent on the fishes' digestive capability (Post *et al.*, 1965; Smith, 1971). Complex carbohydrates are poorly digested by most fishes. Proteins, fats, and simple carbohydrates must supply most of the energy. Physiological fuel values determined for mammals have historically been used for fishes. Research has indicated that these values overestimate the value of starch and underestimate the value of protein. Digestibility of raw starch by most of the carnivorous fishes examined is not more than 40 percent. Digestibility of protein varies with the source, but is generally in the range 70 to 90 percent. Physiological fuel values for fishes appear to be about 3.8, 8.0, and 5.0 kcal per gram of the available carbohydrates, fat, and protein, respectively. Diets should contain about 3,000 to 3,600 kcal of metabolizable energy per kilogram, with about 100 mg of well-balanced protein per kilocalorie of metabolizable energy (Smith, 1971, 1975).

PROTEIN AND AMINO ACIDS

Dietary protein requirements for those fishes that have been studied are quantitatively greater than for higher animals (Dupree and Sneed, 1966; Mertz, 1969). Minimum protein requirements in fish diets are dependent upon the profile and availability of the amino acids in each protein-bearing ingredient. Therefore, care must be exercised in choosing protein sources for fish diets (Halver, 1972b; Ogino and Saito, 1970; Phillips, 1970).

Most of the research on general protein requirements of fishes has been done with rainbow trout, chinook salmon, and channel catfish. Mathematical expression of these requirements is inverse to the size or age within a species, for example, 56 to 38 percent of the diet as protein for chinook salmon, 40 to 32 percent for rainbow trout, and 36 to 28 percent for channel catfish. Therefore, the protein quality and digestibility of each ingredient must be relatively high in order to supply necessary quantities of each indispensable amino acid. Reduced protein quality should be compensated by increased quantities of protein or by supplementing with amino acids into finished diets (Rumsey and Ketola, 1975). The above ranges can be used as a guide for the protein requirements of other fishes, because only cursory research has been done on their quantitative protein needs (Cowey and Sargent, 1972; Halver, 1972b).

An amino acid test diet has been used to establish the qualitative and quantitative amino acid requirements of a limited number of fish species (Arai *et al.*, 1971; Halver, 1957). Findings of this research indicated that 10 amino acids are indispensable for salmon, trout, catfish, and eel (Dupree and Halver, 1970; Halver *et al.*, 1957; Nosé, 1969). Dietary quantities of each of these amino acids have also been established for chinook salmon (Table 26). These same quantities are acceptable for trout, catfish, and certain other fish species.

Two amino acids can act to spare two indispensable amino acids (cystine spares methionine and tyrosine spares phenylalanine, Table 26). A relationship between quantities of leucine and isoleucine appears necessary for most satisfactory fish growth (Table 26). A small increae of isoleucine in the diet demands a much greater increase of leucine (Chance *et al.*, 1964).

Diets composed of poor-quality protein, or deficient in protein, will result in poor growth and mortality in extreme instances. Marked alteration of amino acid balance, or limitations of an essential amino acid, will result in reduced growth rate and subsequent mortality. Limiting quantities of any single amino acid will result in reduced growth of fish (Halver, 1972b).

Pathological conditions derived from deficiencies of an in-

TABLE 26 Amino Acid Requirements of Chinook Salmon

Amino Acid	Percent of the Diet
L-arginine	2.4
L-histidine	0.7
L-isoleucine	0.9 or 1.0
L-leucine	1.6 or 3.3
L-lysine	2.0
L-methionine	1.5[a]
L-phenylalanine	2.1[b]
L-threonine	0.9
L-tryptophan	0.2
L-valine	1.3

[a] Up to two-thirds of methionine may be supplied by cystine.

[b] Up to one-fifth of phenylalanine may be supplied by tyrosine.

SOURCES: Chance *et al.*, 1964; DeLong *et al.*, 1958; NRC, 1973; Post, 1972.

dispensable amino acid do not occur, except for tryptophan (Halver, 1972a). Tryptophan-deficient fishes develop scoliosis and, to a limited degree, lordosis. The condition is transitory, and fishes recover normal activity and appearance within 2 weeks after addition of a sufficient quantity of tryptophan to the diet (Kloppel and Post, 1975).

FATS

Dietary fats are a major energy source for fishes that have been studied. Natural diets of certain fishes may contain up to 20 percent fats, characteristically composed of highly unsaturated fatty acids with chain lengths of 20–22 carbons predominating. Certain of the fatty acids (linolenic family, ω3) are essential to health, growth, and normal appearance of fishes (Castell *et al.*, 1972; Lee and Sinnhuber, 1972; Sinnhuber, 1969).

Fats for fish diets should be limited to the more unsaturated fats. Hard (saturated) fats are poorly digested and utilized by fishes, especially the coldwater and coolwater fishes (Phillips, 1970).

The total fat content of a fish diet is somewhat limited by the procedure used for diet preparation. Moist diets can have a fat content as high as 16–20 percent, if care is taken to use unsaturated fats stabilized by antioxidants (Crawford *et al.*, 1973). Dry particulate or pelleted diets are generally limited to no more than 10–11 percent fat because of the fluid nature of the fats and the necessity for binding the solid particles. Most commercial fish diets have fat contents that range between 3 and 15 percent (Huet, 1973).

Careful attention must be given to presence of the necessary quantities of the essential fatty acids. Trout require about 1 percent of the diet as 18:3 ω3 (linolenic series) fatty acid; approximately 0.1 percent of this quantity may be supplied by 18.3 ω6 fatty acid (Sinnhuber, 1969). Best sources of 18:3 ω3 fatty acid are fish oils (menhaden oil averages about 30 percent, salmon oil about 27 percent, and herring oil about 21 percent). Vegetable oils are generally low in this fatty acid (corn oil averages about 1 percent, safflower oil about 0.3 percent, and raw soybean oil about 7 percent) Cowey and Sargent, 1972; Kayama, 1964; NRC, 1973).

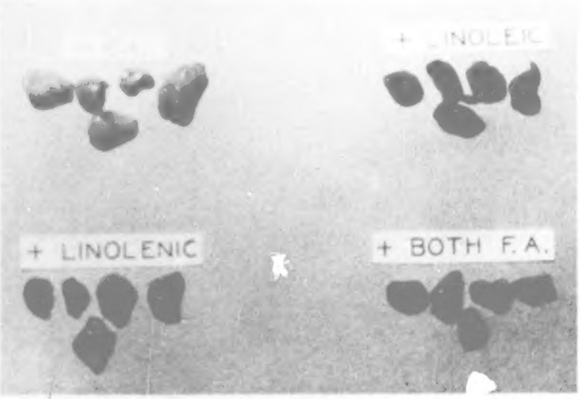


FIGURE 2 Livers from brook trout fed semipurified diets supplemented with either, or both, linoleic and linolenic acids. Deficiency results in creamy white-colored liver with altered fatty acid composition. (Photographs by Poston)

Unsaturated fats in fish diets make the presence of an antioxidant essential for satisfactory storage. Storage of fish diets at low (refrigerator) temperature and humidity may retard oxidation of these fats and increase the time the diet may be held in a satisfactory condition before feeding (NRC, 1973).

Deficiency of the linolenic family (ω3) fatty acids, will lead to several pathological disorders (Figure 2). Reduced body phospholipid and depigmentation have been described by Sinnhuber (1969) and Lee and Sinnhuber (1972). Rancid or peroxidized fats can impair liver and kidney function with resultant mortality. A general unthriftiness and darkened appearance is often noted. Cyclopropenoid fatty acids, which occur naturally in some plant protein concentrates and oils, should be avoided, as they decrease growth and act as cocarcinogens (NRC, 1973).

CARBOHYDRATES

Intermediary metabolism of carbohydrates is controlled by the same enzyme systems in fishes as those found in higher animals. Studies of carbohydrate metabolism in fishes have demonstrated that digestibility of these substances is lower than in higher animals (Buhler and Halver, 1961; Nagai and Ikeda, 1971).

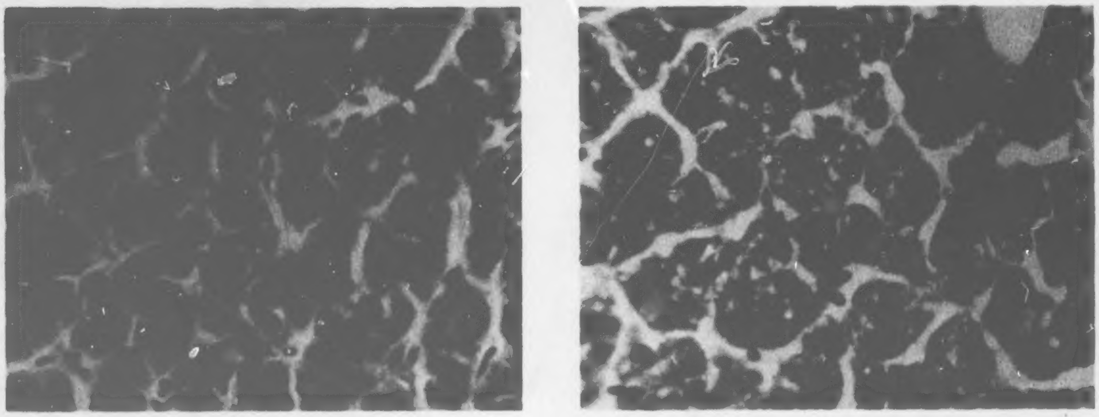
Configuration of certain carbohydrates affects digestibility. Raw starch is less available than gelatinized starch (Phillips and Brockway, 1956). Formulation of fish diets requires close attention to the specific sugars or starches present in each ingredient. Approximate availability of total carbohydrate from a diet can be calculated. Maximum digestible carbohydrate should not exceed 20 percent (Table 27). Digestible carbohydrate should not be equated to total nitrogen-free extract (Post, 1972).

Excess digestible carbohydrate increases blood sugar, which in most fishes ranges from 70 to 120 mg per 100 ml of blood, and increases liver glycogen. Normal fishes have from 0.5 to 3.0 percent liver glycogen. Excessive digestible

TABLE 27 Digestibility of Certain Carbohydrates for Trout

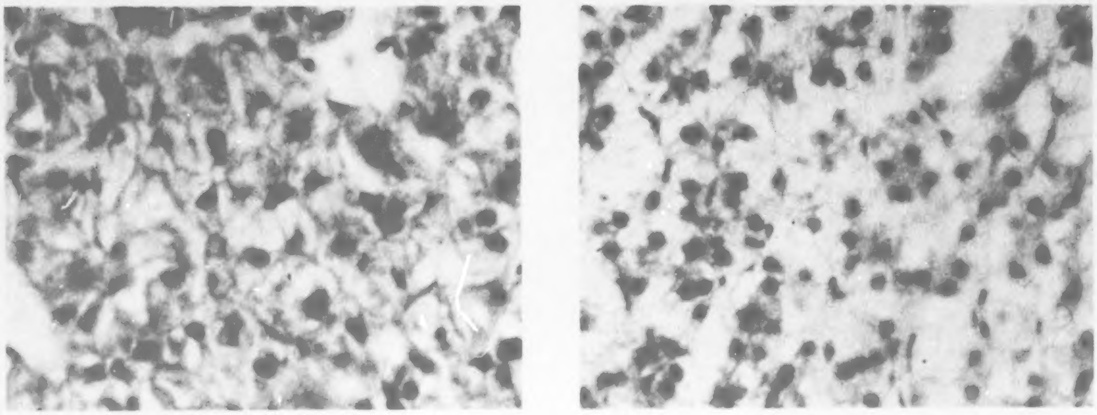
Carbohydrate	Percent Digestibility
Glucose	90–99
Glycogen	90–99
Maltose	92
Dextrin	80
Sucrose	73
Lactose	60
Starch (gelatinized)	57–70
Starch (raw)	38
α-Cellulose	10

SOURCES: NRC, 1973; Phillips and Brockway, 1956.



2% Liver Glycogen
(Normal)

6% Liver Glycogen
(Moderate Hyperglycogenesis)



9% Liver Glycogen
(Marked Hyperglycogenesis)

14% Liver Glycogen
(Extreme Hyperglycogenesis)

FIGURE 3 Feeding of high concentrations of digestible carbohydrate to fishes results in hyperglycogenesis and excessive glycogen storage in the liver. Liver function may become increasingly impaired as liver glycogen increases because of vital cellular structures replacement with glycogen. Magnified 800 times. (Photographs by Post)

carbohydrate may increase this level to greater than 17 percent, which leads to liver-function impairment. The result is liver hyperglycogenesis (Figure 3) and nephrosis. Liver glycogen concentrations of more than 17 percent may either cause mortality directly or contribute to mortality from other causes. Total liver mass may also increase as liver glycogen increases. Most trout have a liver mass between 0.8 and 1.2 percent of the body weight and liver hyperglycogenesis may cause this ratio to be exceeded (Post, unpublished). Other fish species may have a similar liver mass to body weight ratio.

BULK (FIBER)

Only limited research on bulk requirements of fishes has been accomplished. Studies with channel catfish, which have a recurved intestine, indicate that diets containing 10 to 20 percent crude fiber had beneficial effects on growth when compared to diets containing lesser amounts (Dupree and Sneed, 1966). In contrast, bulk in trout diets may reduce absorption of other nutrients because of relatively rapid food passage through the short, straight intestine. Fiber content for salmonid diets should be 4 percent or less.

TABLE 28 Vitamin Requirements for Fishes

Vitamin	Requirements (in mg/kg body weight per day)[a]	Recommended mg/kg of Ration
Water-soluble		
Ascorbic acid	3–5	100
B_{12}	0.0002–0.0003	0.02
Biotin	0.03–0.07	1
Choline	50–60	3,000
Folacin (folic acid)	0.15–0.20	5
Inositol	18–20	400
Niacin (nicotinic acid)	3–7	150
Pantothenic acid	1.0–1.5	40
Pyridoxine	0.2–0.4	10
Riboflavin	0.5–1.0	20
Thiamin	0.15–0.20	10
Fat-soluble		
A	60 IU	2,000 IU
E	1 IU	30 IU
K	2 IU	80 IU
D[b]		

[a] Based on young fishes.

[b] Requirement for vitamin D has not been established in fishes.

VITAMINS

Rearing or holding fishes in water systems that are devoid of natural food requires that diets have all essential vitamins in satisfactory quantities to support the needs of the fish (Dupree, 1966). Much of the research on vitamin requirements of fishes has been done on salmon, trout, channel catfish, and, to a limited extent, on carp and eels (Aoe *et al.*, 1971, 1967; Halver, 1969, 1972b; Halver and Coates, 1957, NRC, 1973). These studies yielded information on daily requirements of each vitamin (Table 28) and can be used to formulate diets (Post, 1972).

Specific vitamin deficiency signs are given in Table 29 and illustrated in Figures 4 through 8. The only hypervitaminoses known in fishes occur with fat-soluble vitamins. Hypervitaminosis A results in reduced growth, lowered hematocrit, and necrosis of the caudal fin (Poston *et al.*, 1973). However, excessive concentrations of all vitamins should be avoided in fish diets (NRC, 1973).

TABLE 29 Specific Vitamin Deficiency Signs

Vitamin	Deficiency Signs
Water-soluble	
Ascorbic acid	Scoliosis; lordosis; impaired collagen formation; altered cartilage; capillary fragility
B_{12}	Hematologic disorders; fragile erythrocytes; poor growth
Biotin	Skin lesions (blue slime); muscle atrophy; spastic convulsions; erythrocyte fragility; poor growth
Choline	Poor growth; poor food conversion; hemorrhagic kidney and intestines; accumulation of neutral fats in liver
Folacin	Poor growth; lethargy; fragile caudal fin; dark coloration; macrocytic anemia
Inositol	Poor growth; distended stomach; increased gastric emptying time
Niacin	Loss of appetite; lesions in colon; muscle spasms while resting; anemia and hemorrhage in skin; skin lesions
Pantothenic acid	Clubbed gills; loss of appetite; poor growth; exudate on gills
Pyridoxine	Nervous disorders (epileptiform convulsions); anemia; loss of appetite; edema in peritoneal cavity; blue-violet iridescent skin color; rapid rigor mortis; rapid and gasping breathing; flexing of opercles
Riboflavin	Corneal vascularization; cloudy lenses; hemorrhagic eyes; reduced vision; abnormal pigmentation of iris; dark coloration; anemia; poor growth
Thiamin	Convulsions followed by body flexure and possibly death; instability and loss of equilibrium; edema; poor growth
Fat-soluble	
A	Retinal alterations; poor growth
D	No specific signs
E	No specific signs (may mimic other vitamin deficiencies, especially of those vitamins easily oxidized, and are protected by the antioxidizing activity of vitamin E)
K	Reduced blood-clotting time

SOURCES: Halver, 1972a; Hashimoto, 1972; Hashimoto and Okaichi, 1969; NRC, 1973.

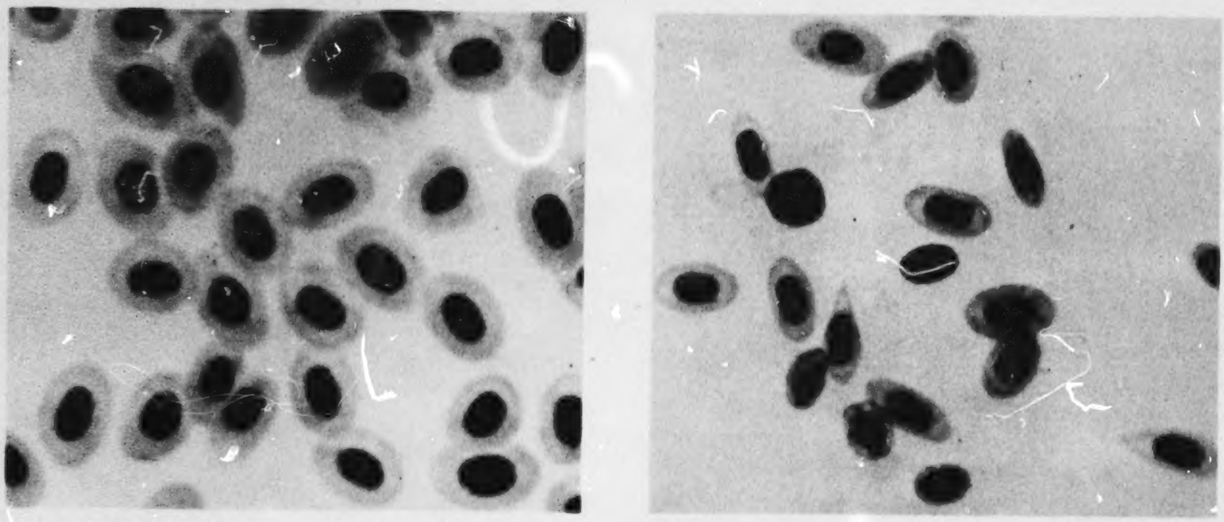


FIGURE 4 Fish erythrocytes are normally oval in shape, with pronounced oval nucleus and cytoplasm of approximately equal volume (left photograph). Folic acid-deficient fish (right photograph) erythrocytes usually are of unequal size with occasional bizarre nuclei and are reduced in number and hemoglobin content. Both photographs are magnified 3,000 times. (Photographs by Post)

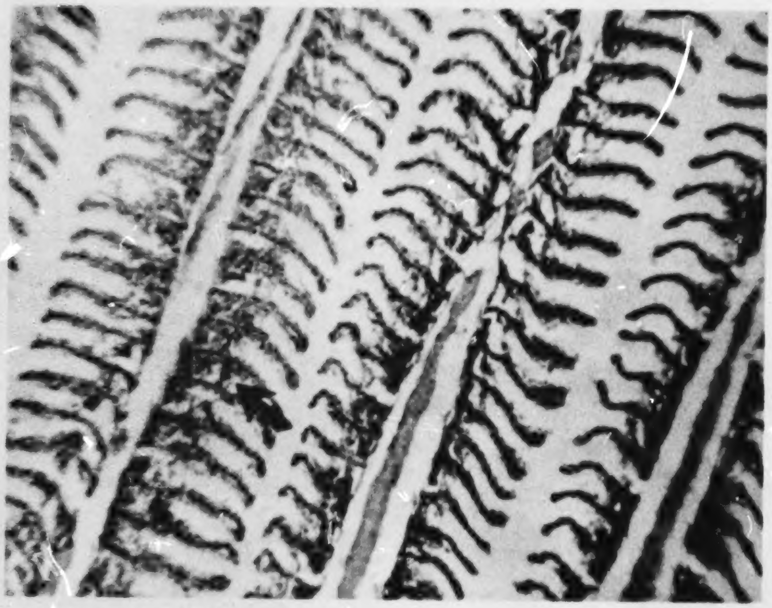


FIGURE 5 Pantothenic acid deficiency causes basal (proximal) hyperplasia of gill lamellae (arrow) as an early development of the deficiency. Advanced pantothenic acid deficiency may involve the entire lamellae and gill filaments. Magnified 100 times. (Photograph by Post)

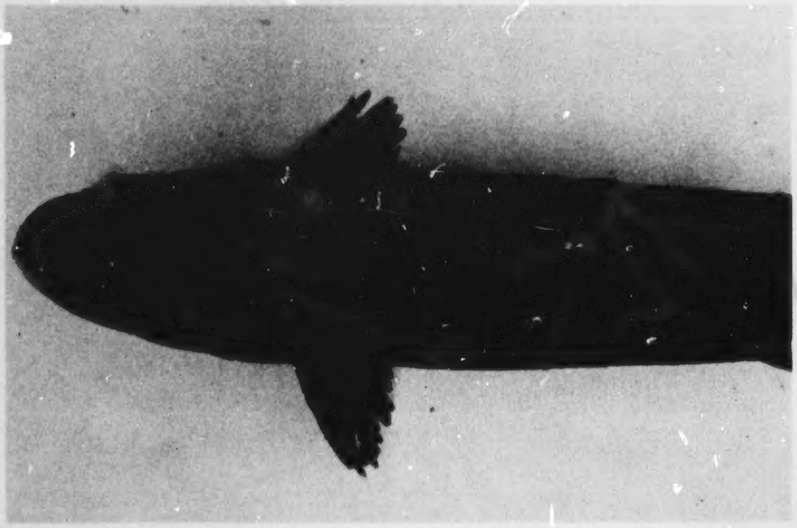


FIGURE 6 Niacin deficiency causes loss of scales, sloughing of underlying epithelial cells, frayed appearance of fins, and erosion of underlying muscle tissues in advanced cases. Secondary fungal invasion may occur. (Photograph by Post)

MINERALS

The exchange of mineral ions across the gill membrane and the skin of fishes reduces the dietary needs for those that are absorbed from the alimentary tract (Phillips, 1959). Presence of required amounts of the most diffusible ions in water (chloride, carbonate, sodium, potassium, phosphorus, calcium, magnesium, and others) reduces the absorption of these ions from the diet.

Iodine is a gill-diffusible element. However, certain natural waters of the world are deficient in it. Therefore, iodine is usually incorporated at 0.6 to 1.1 mg per kilogram of diet (NRC, 1973; Woodall and LaRoche, 1964).

Those mineral elements necessary for health in other animals are required by fish. Many, such as iron, cobalt, sulfur, phosphorus, copper, and others are usually added to fish diets in order to assure an adequate supply of each (Ketola, 1975b). The mixture given in Table 30 should fulfill all mineral requirements of fishes fed natural-ingredient diets. More minerals, and generally larger quantities, must be added to purified diets (Table 31).

Deficiency of minerals in water or in the diet may cause specific signs. A deficiency of iodine causes thyroid hyperplasia or goiter, which will regress on addition of this element to the diet (Woodall and LaRoche, 1964). Calcium and phosphorus, in addition to osmoregulatory function, are necessary for bone mineralization. Selenium has been demonstrated to be important in prevention of white muscle disease or muscular dystrophy (Poston *et al.*, 1976). Deficiencies of cobalt and iron result in impaired growth or depressed hematocrits (Halver, 1972a,b).

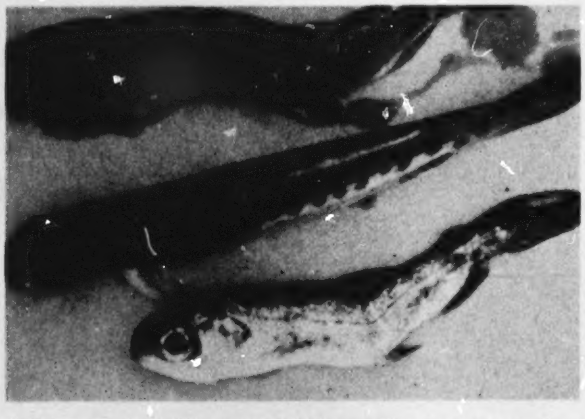


FIGURE 7 Ascorbic acid deficiency. The top and bottom coho salmon are deficient in ascorbic acid and show typical scoliosis and lordosis. The middle fish is normal. (Photograph by Rumsey)

TABLE 30 Mineral Mixtures for Use in Natural-Ingredient Diets

Mineral	Grams per 100 g of Diet
Coldwater fish diet	
NaCl	0.500
$MgSO_4$	0.200
$MnSO_4 \cdot H_2O$	0.100
$ZnSO_4 \cdot H_2O$	0.030
$FeSO_4 \cdot 7H_2O$	0.030
$CuSO_4$	0.030
KIO_3	0.0009
Warmwater fish diet	
$CaCO_3$	0.075
NaCl	0.075
$ZnSO_4 \cdot 7H_2O$	0.070
$FeSO_4 \cdot 7H_2O$	0.050
$MnSO_4 \cdot H_2O$	0.030
$CuSO_4 \cdot 5H_2O$	0.006
KIO_3	0.0002

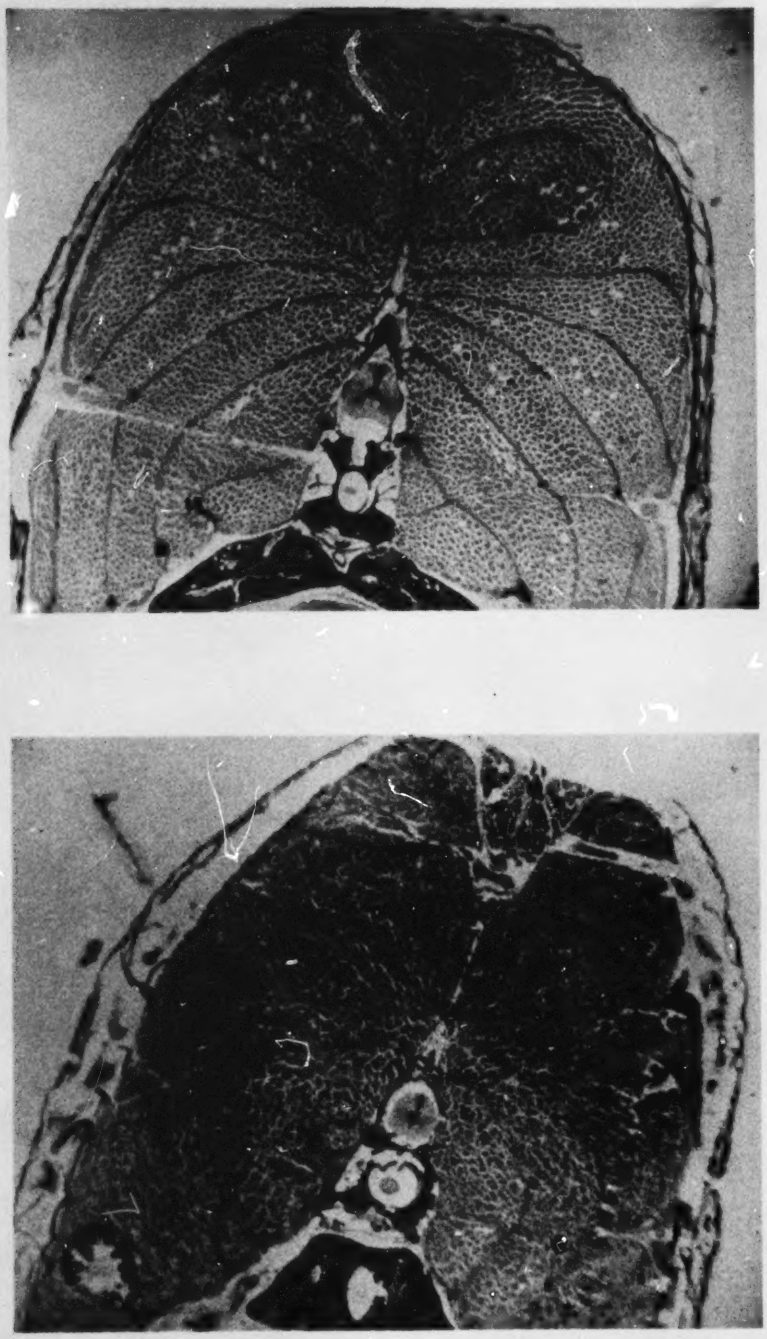


FIGURE 8 Transverse section of dorsal musculature of control fish (upper photograph) and fish with nutritional muscular dystrophy (lower photograph) after 16 weeks of dietary vitamin E and selenium deficiency. Dystrophic fish is characterized by loss of the definition of muscle bundles shown in normal or control fish. Magnified 10 times. (Photograph by Poston)

TABLE 31 Mineral Mixture for Use in Purified Diets

Mineral	Grams per 100 g of Diet
$CaHPO_4 \cdot 2H_2O$	2.07
$CaCO_3$	1.48
KH_2PO_4	1.00
NaCl	0.60
$MgSO_4$	0.30
KCl	0.10
$FeSO_4 \cdot 7H_2O$	0.05
$MnSO_4 \cdot H_2O$	0.035
$ZnCO_3$	0.015
$CuSO_4 \cdot 5H_2O$	0.003
KIO_3	0.001
$NaMoO_4 \cdot 2H_2O$	0.00083
$CoCl_2$	0.00017
Na_2SeO_3	0.00002

ADVENTITIOUS TOXINS

Toxic materials that can affect fishes are mycotoxins, naturally occurring plant toxins, pesticides, industrial contaminant residues, and heavy metals such as lead and mercury. Fishes are extremely sensitive to mycotoxins, and great care should be taken to avoid mold-contaminated ingredients (Figure 9) (Liener, 1969; NRC, 1973; Wogan, 1966). Toxins and nutrient inhibitors occurring naturally in plant materials, and more specifically in oilseed meals, can usually be destroyed by proper processing (e.g., heating, chemical treatment, or extraction). Fishes seem to be especially sensitive to the soybean trypsin inhibitor, and special precautions must be taken with diets that may contain it (Sandholm *et al.*, 1976). Ingredients should be examined for toxic residues when and where there is a possibility that contamination will compromise experimental results.

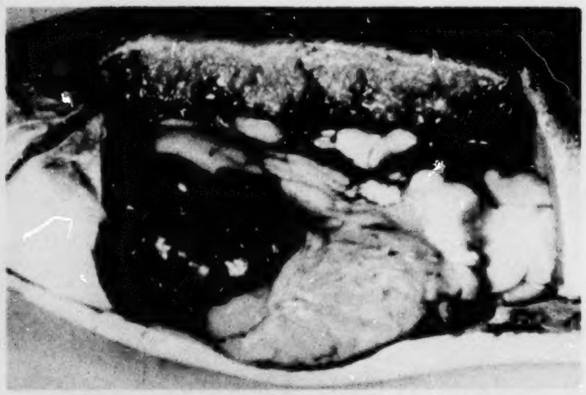


FIGURE 9 Rainbow trout with hepatoma. This fish was fed 20 ppb aflatoxin for 9 months. (Photograph by Halver)

TABLE 32 Purified Diet for Use in Studies with Coldwater and Warmwater Growing Fishes

	Percent of Dry Diet	
Ingredient	Coldwater Fishes	Warmwater Fishes
Casein	35	28
Dextrin	30	28
Gelatin	15	12
Soybean oil and/or fish oil	9	9
α-Cellulose	6	18
Mineral mixture[a]	4	4
Vitamin mixture[b]	1	1

[a] Table 31.
[b] Table 33.
SOURCES: Dupree, 1966; Halver and Coates, 1957.

DIET CONSIDERATIONS AND FEEDING PRACTICES

A primary consideration in devising a formula is to ascertain how the food will be used. The diet may be needed for alevins, fingerlings, or fishes held for reproductive products (Hunt, 1973).

Purified and natural-ingredient diets for growing warmwater and coldwater fishes are described in Tables 32, 33, 34, and 35. The formulas described are designed for actively growing fishes and must be adjusted accordingly for the other various life stages.

The diets may be fabricated into moist (approximately 70 percent water), semimoist (approximately 35 percent water), and dry forms (approximately 10 percent water).

TABLE 33 Vitamin Mixture for Use in Purified Diets

Vitamin	Amount per 100 g of Diet[a]
Choline·Cl, mg	450.0
Niacin, mg	100.0
Inositol, mg	20.0
Ascorbic acid, mg	15.0
Vitamin K,[b] mg	12.0
Calcium pantothenate, mg	6.0
Pyridoxine, mg	1.5
Riboflavin, mg	1.5
Thiamin·HCl, mg	1.5
Antixoidant,[c] mg	1.0
Folacin (folic acid), mg	0.5
Biotin, mg	0.15
Vitamin B_{12}, mg	0.003
Vitamin A, IU	500.0
Vitamin D_3, IU	200.0
Vitamin E, IU	5.0

[a] These quantities added to α-cellulose to make 1 percent of the diet.
[b] Menadione dimethylpyrimidinol bisulfite.
[c] Butylated hydroxytoluene (BHT) and/or ethoxyquin.

TABLE 34 Natural-Ingredient Diets for Use in Studies with Coldwater and Warmwater Fishes

	Percent of Dry Diet	
Ingredient	Coldwater Fishes	Warmwater Fishes
Fish meal (60% protein)	45	10
Blood meal	—	10
Soybean meal (50% protein)	10	20
Corn gluten meal (60% protein)	10	—
Dried distiller's solubles	5	10
Cottonseed meal (49% protein)	—	10
Rice bran	—	21
Wheat middlings	15	10
Dehydrated alfalfa meal	—	4
Brewer's dried yeast	5	—
Dried whole whey	5	—
Fish or soybean oil	3	3
Vitamin mixture[a]	1	1
Mineral mixture[b]	1	1

[a] Table 35.
[b] Table 30.

The diets will float or sink, depending on the processing technology used, e.g., conventional pressurized pelleting versus extrusion-expansion. The use of varying moisture levels, as well as a floating or sinking pellet, is the choice of the researcher; little difference is noted in the fishes (Phillips, 1970; Poston, 1974). Excessive heat required to make expanded pellets can be detrimental to labile nutrients in the diet. Costs also are important, as the pressurized or compressed sinking pellet is less expensive to make than the extruded-expanded variety. The dry form is recommended, as it can be held for a longer time without refrigeration and does not require special storage facilities. Diets should be carefully selected by the investigator and the fishes maintained on the same batch of diet throughout the course of the experiment (Neuhaus and Halver, 1969).

TABLE 35 Vitamin Mixture for Use in Natural-Ingredient Diets

Vitamin	Amount per 100 g of Diet[a]
Choline·Cl, mg	100.0
Vitamin K,[b] mg	12.0
Antioxidant,[c] mg	10.0
Ascorbic acid, mg	10.0
Thiamin·HCl, mg	5.5
Niacin (nicotinic acid), mg	5.0
Calcium pantothenate	2.0
Folacin (folic acid), mg	0.5
Pyridoxine, mg	0.5
Riboflavin, mg	0.5
Biotin, mg	0.1
Vitamin B_{12}, mg	0.001
Vitamin A, IU	500.0
Vitamin D_3, IU	200.0
Vitamin E, IU	5.0

[a] These quantities added to an inert ingredient to make 1 percent of the diet.
[b] Menadione dimethylpyrimidinol bisulfite.
[c] Butylated hydroxytoluene (BHT) and/or ethoxyquin.

Care should be exercised in the selection of ingredients to be used in a test diet. Variations in the nutrient composition and properties of individual ingredients occur between lots and sometimes within the same lot. Special attention and precaution should be directed to potential toxic residue contamination as typified by organochlorines.

The particle size and homogeneity of the fish diet are critical considerations (Huet, 1973; Phillips, 1970). This is especially so for young fishes with small mouth parts. Particle size recommendations for various sized trout have been established and can serve as general guides in feeding other fishes (Table 36).

The amount of diet that fishes should be fed daily in order to achieve optimum utilization is related to species, fish size, diet quality, and water temperature (Huet, 1973). A guide to quantities of ration in percent of body weight to be fed to trout was given by Deuel *et al.* (1952). Trout species, body size, and environmental temperature were considered. If a feeding guide is not used to determine daily diet quantities, fishes should be fed slightly less than maximum consumption to minimize waste. Reliable figures for energy requirements of all species have not been established. However, nutritionists agree that conversion ratios of food to fish tissue should be 2 or less.

There is little experimental evidence upon which to establish the number of feedings that fishes should receive per day. Most information available involves salmonids and can be used as a guide to the number of feedings during a working day: more than 1,100 fish/kg should be fed at least eight times per day; between 1,100 and 330 fish/kg, five times per day; between 330 and 200 fish/kg, three times per day, providing the water temperature is above 8°C. The foregoing schedule is acceptable when fishes are uniform in size and food can be distributed evenly among individuals. There is reason to believe that increasing feeding frequency leads to more uniformity in fish size. Fishes should be fed 7 days a week (Huet, 1973; Phillips, 1970).

TABLE 36 Food Particle Size Recommendations for Trout

Pellet or Granule Size		U.S. Series Sieve Opening	U.S. Series Standard No.	Fish Size (g) Begin	Fish Size (g) End
Starter	Through	595 microns	30	—	up to 0.2
	Over	420 microns	40		
#1 Granule	Through	841 microns	20	0.2	0.5
	Over	595 microns	30		
#2 Granule	Through	1.19 mm	16	0.5	1.8
	Over	841 microns	20		
#3 Granule	Through	1.68 mm	12	1.8	4.5
	Over	1.19 mm	16		
#4 Granule	Through	2.83 mm	7	4.5	15.0
	Over	1.68 mm	12		
	Diameter		Length		
3.18-mm pellet	3.18 mm	×	3.18 mm	15	45
4.76-mm pellet	4.76 mm	×	4.76 mm	45	150
6.35-mm pellet	6.35 mm	×	6.35 mm	150	—

SOURCE: Adapted from Phillips, 1970.

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